Implications of BRCA1 mutations in basal-like breast cancer development and treatment

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Academic dissertation

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences, University of Helsinki, in Biomedicum Helsinki 1 (Haartmaninkatu 8), Lecture Hall 2, on June 16, 2015, at 12:00 noon.

Helsinki 2015

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Published in Dissertationes Scholae Doctoralis Ad Sanitatem Investigandam Universitatis Helsinkiensis
ISBN 978-951-51-1195-1 (paperback)
ISBN 978-951-51-1196-8 (PDF)
ISSN 2342-3161 (Print)
ISSN 2342-317X (Online)

Cover layout by Anita Tienhaara
Cover image: Breast cancer awareness ribbon woman head composite.
Hansaprint Oy, Vantaa, Finland 2015

If you don't know your future, then go for it.

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List of original publications

The thesis is based on the following articles referred to in the text by their Roman numerals:

I. Gu Y, Bouwman P, Greco D, Saarela J, Yadav B, Jonkers J, and Kuznetsov SG (2014) Suppression of BRCA1 sensitizes cells to proteasome inhibitors. Cell Death Dis 18;5:e1580.

II. Munne PM, Gu Y, Tumiati M, Gao P, Koopal S, Uusivirta S, Sawicki J, Wei G, and Kuznetsov SG (2014) TP53 supports basal-like differentiation of mammary epithelial cells by preventing translocation of deltaNp63 into nucleoli. Sci Rep 4:4663.

III. Yuexi Gu, Kristiina Väänänen, Jani Saarela, Anna Sokolenko, John Martens, Evgeny Imyanitov, and Sergey Kuznetsov. BRCA1-deficient breast cancer cell lines are resistant to MEK inhibitors and show distinct sensitivities to 6-thioguanine. *Manuscript*.

The author's contribution to each publication:

I. Y.G. conceived the idea of carrying out a high-throughput chemical compound screen on a pair of BRCA1- and control-knockdown MDA-MB-231 cells, which is originally a basal-like breast cancer cell line expressing wildtype BRCA1. Y.G. designed and perfomed most of the experiments, analyzed the data, and wrote the manuscript together with S.K.

II. Y.G. helped to perform several experiments of Western blotting and reverse transcription–quantative PCR (RT-qPCR) that are related to BRCA1.

III. Y.G. performed most of the experiments under the supervision of S.K., analyzed the data and helped to write the manuscript.

Abbreviations

53BP1	p53-binding protein 1
ATM	ataxia telangiectasia mutated
ATR	ataxia telangiectasia mutated and Rad3-related protein
ATRIP	ATR-interacting protein
BARD1	BRCA1-associated RING domain protein 1
BER	base excision repair
BLM	Bloom syndrome protein
BRCA1	breast cancer 1, early onset
BRCA2	breast cancer 1, early onset
BRCC36	BRCA1/BRCA2-containing complex, subunit 36
BRCC45	BRCA1/BRCA2-containing complex, subunit 45
BRCT	BRCA1 C terminus
CHK1	checkpoint kinase 1
CHK2	checkpoint kinase 2
CtBP	C-terminal-binding protein
CtIP	CtBP-interacting protein
DNA2	DNA replication helicase/nuclease 2
DNA-PKcs	DNA-dependent protein kinase, catalytic subunit
DSB	double-strand break
EGFR	epidermal growth factor receptor
EME1	crossover junction endonuclease
EMT	epithelial-to-mesenchymal transition
ER	estrogen receptor
ERCC1	excision repair cross-complementation group 1
EXO1	exonuclease 1
H2AX	H2A histone family, member X
HER2	human epidermal growth factor receptor 2 (ERBB2)
HNPCC	hereditary nonpolyposis colorectal cancer
HR	homologous recombination
LOH	loss of heterozygosity
MAPK	mitogen-activated protein kinase
MDC1	mediator of DNA damage checkpoint 1
MEK	mitogen-activated protein kinase kinase (MAPKK, MAP2K)
MGMT	methylguanine methyltransferase
mMEC	mouse mammary epithelial cell
MMEJ	microhomology-mediated end joining
MMR	mismatch repair
MNNG	N-methyl-*N'*-nitro-*N*-nitrosoguanidine
MRN	MRE11-RAD50-NBS1

Abbreviations

MSI	microsatellite instability
MUS8	ubiquitin-conjugating enzyme E2 2
NER	nucleotide excision repair
NHEJ	non-homologous end joining
NLS	nuclear localization signal/sequence
OGG1	8-oxoguanine DNA glycosylase
PALB2	partner and localizer of BRCA2
PARP	poly (ADP-ribose) polymerase
PCNA	proliferating cell nuclear antigen
PI3K	phosphatidylinositol-4, 5-bisphosphate 3-kinase
PNKP	polynucleotide kinase 3'-phosphatase
PR	progesterone receptor
PTEN	phosphatase and tensin homolog
Rb	retinoblastoma protein
RING	really interesting new gene
RNF168	ring finger protein 168, E3 ubiquitin protein ligase
RNF8	ring finger protein 8, E3 ubiquitin protein ligase
RNS	reactive nitrogen species
ROS	reactive oxygen species
RPA	replication protein A
SLX	structure-specific endonuclease subunit
SSB	single-strand break
SSBR	single-strand break repair
STAT	signal transducer and activator of transcription
TBST	tris-buffered saline and Tween 20
TFIIH	transcription factor II Human
TMZ	temozolomide
TNBC	triple-negative breast cancer
WRN	Werner syndrome ATP-dependent helicase
XP	xeroderma pigmentosum
XRCC	X-ray repair cross complementing

Summary

Women with germline mutations of *BRCA1* gene are predisposed to breast and ovarian cancer development. Breast tumors associated with *BRCA1* mutations tend to fall into the basal-like subgroup, which is characterized by no expression of ER, PR and HER2. BRCA1 plays essential roles in DNA damage repair through homologous recombination pathway and, hence, its mutation predicts increased sensitivity to certain DNA-damaging agents, e.g. PARP inhibitors and cisplatin. Yet in clinical trials, some BRCA1-mutant breast tumors are resistant to these agents; and others gain resistance after initial responsiveness. Therefore, it is necessary to understand the molecular mechanisms of the acquired resistance and identify novel therapeutic targets for the treatment of breast cancers caused by *BRCA1* mutations.

Breast cancer cell lines generally have similar behaviors to the tumors from which they were established. In order to better understand the biology of breast cancers caused by *BRCA1* mutations, we collected and characterized four BRCA1-mutant breast cancer cell lines, namely HCC1937, MDA-MB-436, SUM1315MO2 and SUM149PT. Together with several cell lines expressing wildtype BRCA1, we tested their sensitivities to a panel of DNA-damaging agents, including cisplatin and PARP inhibitors. We also carried out a high-throughput screen on the BRCA1-mutant and BRCA1-wildtype cell lines with a library of 198 cancer-related drugs, and performed a proteome profiling assay to test the kinase activities of those cell lines. Our results reveal extensive heterogeneity among the BRCA1-mutant breast cancer cell lines, which showed resistance to DNA-damaging agents. The high-throughput screen demonstrates that the BRCA1-mutant cell lines are more resistant to MEK inhibitors than the BRCA1-wildtype ones.

The heterogeneous phenotypes among those breast cancer cell lines are likely attributed to their distinct genetic backgrounds in addition to different *BRCA1* mutations. To investigate the consequences related only to loss of BRCA1 functions, we created isogenic MDA-MB-231 cells with or without BRCA1 depletion by siRNA transfection. Then we carried out a high-throughput chemical compound screen on the pair of cells in order to identify potential targets that are synthetically lethal with BRCA1 deficiency. Two proteasome inhibitors, bortezomib and carfilzomib, were found to be able to selectively kill BRCA1-depleted cells. In contrast, cells depleted for BRCA2 were not more sensitive to the proteasome inhibitors. This suggests a unique function of BRCA1 other than DNA damage repair in mediating response to proteasome inhibition. Further studies on mechanisms demonstrated that the proteasome inhibitor bortezomib does not induce DNA damage; rather it inactivates G1 cell cycle checkpoint in BRCA1-deficient cells and leads to the accumulation of these cells at G2/M phase. This is caused by inactivation of the retinoblastoma protein (Rb) through its hyperphosphorylation, which in turn activates its

downstream transcription factor E2F1. In addition, bortezomib causes compromised G2/M cell cycle checkpoint in BRCA1-deficient cells, which drives cells to enter mitosis and leads to apoptosis due to uncontrolled cell division. We also found that bortezomib-induced apoptosis in BRCA1-depleted cells is p53 independent but is mediated by 53BP1. Apoptosis of BRCA1-deficient cells caused by bortezomib treatment is inhibited after 53BP1 depletion, which also partially reverses accumulation of these cells at G2/M phase and reactivates Rb.

In order to investigate the mechanisms underlying the consistent basal-like phenotype of breast tumors associated with *BRCA1* mutations, we established an *in vitro* assay to study the transition of mouse mammary epithelial cells from luminal to basal lineages. Our results showed that loss of BRCA1 promotes basal-like differentiation by sustaining ΔNp63 activity. In luminal cells, ΔNp63 is not expressed or remains inactive through localization in nucleoli. Depletion of BRCA1 leads to translocation of ΔNp63 into nucleoplasm and promotes transition of luminal cells into a basal state. This study provides a potential link between BRCA1 loss and the basal-like differentiation, which may help to explain why BRCA1-mutant breast cancer tends to bear a basal-like phenotype.

Introduction

Breast cancer is the most common type of cancer among women worldwide, and accounts for the second highest cause of cancer-related deaths (Siegel et al. 2014). In the past few decades, the overall survival rates of breast cancer patients have increased steadily, thanks to the improvement of diagnosis and treatment. This is partly attributed to molecular stratification of breast cancers according to the expression of key hormonal and growth factor receptors, ER, PR and HER2 (Barton and Swanton 2011). The ER-positive luminal breast tumors can be treated with ER antagonists, while the HER2-overexpressed tumors respond well to the monoclonal antibody trastuzumab (Herceptin) (Olopade et al. 2008). However, at least 15% of breast tumors lack the expression of ER, PR and HER2—referred to as triple negative, and thus cannot be treated with hormonal or anti-HER2 therapies (Olopade et al. 2008). This leaves them with chemotherapy as the only option, which has toxic side effects and is often associated with resistance.

5–10% of breast cancer cases are caused by loss-of-function mutations of certain genes, termed breast cancer susceptibility genes (Ellisen and Haber 1998). *BRCA1* and *BRCA2* are high-penetrance breast cancer susceptibility genes, whose mutations confer up to 80% chance of developing breast cancer in a woman's lifetime (Welcsh and King 2001). Both BRCA1 and BRCA2 proteins are involved in homologous recombination, which is an error-free pathway for the repair of the most deleterious DNA damage, double-strand breaks (DSBs) (Venkitaraman 2009; Roy et al. 2011). As a result, a cell with mutant BRCA1 or BRCA2 relies on error-prone pathways for the repair of damaged DNA, which would lead to genomic instability and eventually development of cancer (Kwei et al. 2010; Negrini et al. 2010). In contrast to breast tumors caused by *BRCA2* mutations, which do not have any consistent phenotypes, BRCA1-mutant breast tumors tend to fall into the basal-like subgroup according to their gene expression profiles (Foulkes et al. 2004; Venkitaraman 2009). Breast tumors from this subgroup are usually triple negative. Hence, the hormonal or anti-HER2 therapies are not beneficial to these patients. On the other hand, based on the functions of BRCA1 in DNA damage repair, it is predicted that BRCA1-mutant breast tumors are highly sensitive to certain DNA-damaging agents, e.g. cisplatin and PARP inhibitors (Turner and Tutt 2012). However, many tumors gain resistance to those therapeutic agents due to secondary mutations of *BRCA1* or other reasons that are still unclear (Lord and Ashworth 2013).

The consistent observations that *BRCA1* mutations lead to breast cancers of a basal-like phenotype suggest that BRCA1 may have multiple functions in preventing cancer development besides DNA damage repair. Indeed, it has been found that, along with its essential roles in homologous recombination, BRCA1 plays pivotal roles in cell cycle and transcriptional regulation, as well as regulating mammary development and stem cell

differentiation (Deng 2006; Mullan et al. 2006; Liu et al. 2008). In this study, we sought to better understand the nature of breast cancers caused by *BRCA1* mutations, for which we characterized several BRCA1-mutant breast cancer cell lines as surrogates of breast tumors. We also carried out a high-throughput chemical compound screen in order to identify potential targets that are synthetically lethal with BRCA1 deficiency. Finally, we established an *in vitro* model to study the differentiation of mouse mammary epithelial cells (mMECs), which would help to uncover the mechanisms underlying the basal-like differentiation of BRCA1-mutant breast cancer.

1. Review of the literature

1.1 Molecular basis of breast cancer

1.1.1 Breast cancer as a major healthcare problem

With the ageing of population in many countries, cancer has become a major healthcare problem around the globe. Among women, breast cancer is the most common invasive cancer; and it is the second leading cause of cancer-related deaths (Siegel et al. 2014). The incidence of breast cancer in developed countries is generally higher than that in developing countries, largely due to their modern lifestyles and longer lifespans. In some western countries, e.g. USA and UK, it is estimated that 1 out 8 women would develop breast cancer in their lifetime. Moreover, the number of breast cancer cases worldwide has significantly increased in the past few decades, thus bringing higher burden to many countries for management.

Breast cancer is associated with multiple risk factors, including some natural factors: gender, aging, genetics, ethnicity; and some environmental factors, e.g. diet, obesity, smoking, exercise, exposure to physical or chemical substances, hormones affected by pregnancy or certain therapies (Eliassen et al. 2006; Veer and Kampman 2007). About 99% of breast cancer cases are diagnosed in women, probably because they have much higher amount of the female hormones, estrogen and progesterone, which can stimulate the growth of the breast tissues (Margolese et al. 2000). Like many other types of cancer, breast cancer incidence is strongly associated with ageing, which might be one of the reasons why nowadays the rates of breast cancer are higher than several decades ago (Margolese et al. 2000). Breast cancer rates are generally higher in western developed countries than that in Asian countries. Even within USA, white women are more likely to develop breast cancer than African- and Asian-American women, highlighting a higher incidence of breast cancer among white populations (Jemal et al. 2011). Various studies have also shown that higher breast cancer incidence is correlated with lower consumption of vegetables, increased amount of alcohol consumed, obesity and lack of exercise (Margolese et al. 2000). Moreover, many breast cancers, especially those diagnosed at relatively young ages, are clearly caused by inherited genetic mutations, e.g. *BRCA1*, *BRCA2* or *TP53* (Apostolou and Fostira 2013).

Treatment of breast cancer depends on stages, types and other factors of the tumors. Most breast cancer patients need surgery to remove the tumors (lumpectomy) or the whole breast (mastectomy) (National Cancer Institute 2014). Surgery is also applied to remove one or both breasts for women at high risks of developing breast cancer later, termed prophylactical mastectomy (Euhus and Diaz 2015). Surgery often is not able to clear all the cancerous cells, so additional therapies (termed adjuvant therapy) are needed to prevent the

relapse of breast cancer. Adjuvant therapies usually involve radiotherapy, chemotherapy, hormone therapy (ER antagonists), targeted therapy (trastuzumab), among other therapies (National Cancer Institute 2014). Despite the steady improvement of overall survival rates of breast cancer patients in the past few decades, treatment of breast cancers of higher stages or certain types remains challenging nowadays. For example, the overall survival rate of stage I breast cancer patients is almost 100%, which goes down to 72% for the stage III patients, but drops dramatically to 22% for the patients with stage IV breast cancer (National Cancer Institute 2015). HER2-overexpressing breast tumors are very aggressive and the overall 5-year survival rate is one third lower than the ER-positive luminal breast tumors (Carey et al. 2006). In addition, accumulated evidence suggests that a large number of breast cancer patients are overtreated, largely due to imprecise diagnosis (Independent UK Panel on Breast Cancer Screening 2012; Alvarado et al. 2011). All of these suggest that more effort needs to be put in studying the mechanisms of breast cancer initiation and progression in order to better guide the treatment.

1.1.2 Different breast cancer subtypes inform on their heterogeneous biology

A healthy female breast contains a mammary gland that is responsible for secreting milk. The mammary gland is made up of 15–20 lobes that produce milk and a network of tubes, called ducts, which carry milk to the nipple (Watson and Khaled 2008). The majority (50–80%) of breast cancers happen in the milk ducts, which are called invasive ductal carcinoma (Weigelt and Reis-Filho 2009). 5–15% of breast cancers are classified as invasive lobular carcinoma, indicating their origin of the lobules. In addition to the two most common types of breast cancer, pathologists have identified many other types of breast cancer with distinctive histological patterns that are associated with consistent clinical outcomes. Currently, at least 17 different types of breast cancer based on histological architectures are recognized by WHO classification (Tavassoli and Devilee 2003). Other relatively common (>1%) types of breast cancer include medullary, neuroendocrine and apocrine carcinomas (Tavassoli and Devilee 2003). The existence of histologically different types of breast cancer, especially those rare ones, not only indicates the heterogeneous biology of breast cancer, but also renders them difficult for molecular studies.

Some breast tumors are aggressive and life-threatening, and they need aggressive treatments that have major adverse effects. These treatments, however, should be avoided for less aggressive tumors to reduce the adverse effects. In order to select the best treatment options, schemes for classifying breast tumors based on their aggressiveness have been developed. These include the TNM (tumor, lymph nodes and metastasis) classification of tumor stages and breast cancer grading (Singletary and Greene 2003). The TNM staging system describes tumor size, degree of spread to regional lymph nodes and presence of

metastasis. Collectively, these parameters classify breast cancers into five stages from 0 to IV, with the overall survival rates decreasing accordingly (Singletary and Greene 2003). The grading of breast cancer scores the parameters of tubule formation, nuclear polymorphism and mitotic count, which altogether classifies breast cancer into three grades (Singletary and Greene 2003).

While these classification systems are widely used by clinicians for early diagnosis, it is more useful to classify breast tumors with certain biomarkers that can help guide treatments and predict prognosis of each group. The hormonal receptors ER and PR, growth factor receptor HER2, and tumor suppressor p53 emerge as important biomarkers for classifying breast cancer along with the histopathological characterization (Malhotra et al. 2010). About two thirds of breast tumors express ER and/or PR, and they are less aggressive than those tumors overexpressing HER2 protein (Ignatiadis and Sotiriou 2013). Some breast tumors do not express any of the ER, PR or HER2 receptors, and hence they are termed triple-negative breast cancer (TNBC) (Foulkes et al. 2010; Turner and Reis-Filho 2013). These tumors almost always have mutated p53, a sign of poor prognosis. It is important to determine the proliferation rate of breast cancer cells during diagnosis, where the proliferation marker Ki-67 is often used to help with classification (Feeley et al. 2013).

Microarray-based gene expression profiling has explored the biology of breast cancer at a deeper level. Such studies demonstrate that breast cancer is not a single disease but a collection of heterogeneous diseases that can be classified into at least four subgroups: luminal A, luminal B, HER2-overexpressing, and basal-like (Figure 1) (Perou et al. 2000; Sørlie et al. 2003; Viale 2012; Cancer Genome Atlas Network 2012). Luminal subtypes of breast cancer have similar gene expression profiles to cancers starting from the luminal layer of mammary ducts (Ignatiadis and Sotiriou 2013). They are mainly positive for ER and/or PR, but differ in their tumor grades. Luminal A breast tumors have lower grades they have the best prognosis among all subgroups of breast cancer, with a low recurrence rate (Sørlie et al. 2001). The relatively high grades of luminal B tumors are determined by several factors: higher ratio of p53 mutations, positive for HER2 expression and highly positive for Ki-67, which altogether contribute to a poorer prognosis and relatively low survival rate (Cheang et al. 2009; Van De Vijver et al. 2002).

HER2-overexpressing breast tumors, as the name implies, express high levels of HER2 protein due to gene amplifications (Hynes and Lane 2005). Overexpression of HER2 activates several cellular signaling pathways, including MAPK, PI3K/Akt and STAT, all of which can stimulate proliferation of the cancer cells (Gajria and Chandarlapaty 2011; Roy and Perez 2009). Tumors from this group usually do not express ER or PR, having high grades and are very aggressive. This subtype of breast cancer has the worst prognosis, and is prone to recurrence and metastasis (Sørlie et al. 2001; Sotiriou et al. 2003).

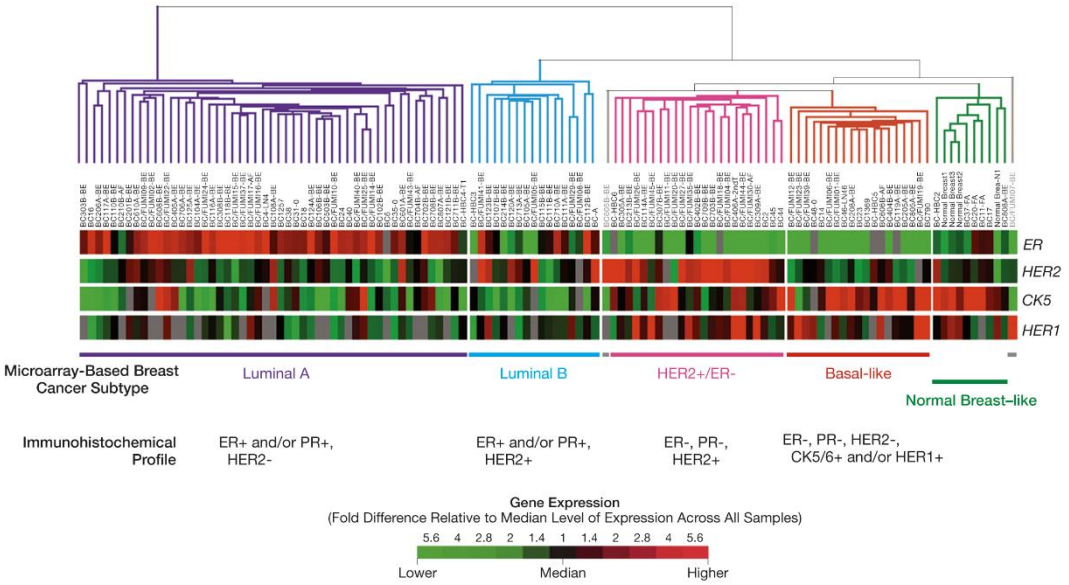

Figure 1. Molecular classification of breast cancer and the associated histological phenotypes. Breast cancer can be classified into different subtypes according to their intrinsic gene expression profiles: luminal breast tumors express ER and/or PR; HER2-overexpressing tumors express high levels of HER2; the basal-like subtype of breast cancer is usually negative with the three receptors. (Reproduced from Lisa et al. 2006, with the permission of JAMA Network)

The basal-like subtype of breast cancer has a gene expression profile similar to the basal/myoepithelial cells of the mammary epithelium (Rakha et al. 2008; Rakha and Ellis 2009). They are characterized by expression of high-molecular-weight cytokeratins, keratins 5/6, 14 and 17, as well as other basal/myoepithelial makers, e.g. EGFR (HER1) and ΔNp63, an isoform of transcription factor p63 that belongs to the p53 family (Figure 1) (Foulkes et al. 2010; Rakha et al. 2008; Gusterson et al. 2005). However, unlike normal basal/myoepithelial cells, most basal-like tumors also express the luminal cell markers keratins 8 and 18, raising questions about the origins of these tumors (Badve et al. 2011; Livasy et al. 2005). In addition, these tumors do not express ER, PR or HER2, and are mutant for p53, so they are largely overlapping with triple-negative breast tumors. Breast tumors from the basal-like subgroup also have high grades with aggressive phenotypes and poor prognosis compared with tumors from the luminal subgroups (Badve et al. 2011; Toft and Cryns 2010).

Despite detailed characterization of each subtype of breast cancer, different studies may have slightly different parameters for classification. For example, about 6–10% of breast cancers are classified as normal-like breast cancer by some studies, since their gene expression profiles resemble normal breast tissues and cannot be classified into any of the subgroups described above (Figure 1) (Rakha et al. 2008; Kapp et al. 2006). Many genes

characteristic of adipose cells are highly expressed in the tumors from this subgroup, while those luminal epithelial cell markers have lower expressions (Rakha et al. 2008). In addition, some studies suggest that another subgroup, claudin low, can be isolated from the basal-like subtype of breast cancer (Herschkowitz et al. 2007). This group accounts for about 10% of all breast cancers and the tumors are characterized by no or low expression of the tight junction proteins of claudin family. Moreover, they have high enrichment of epithelial-mesenchymal transition (EMT) markers and cancer stem cell features, and hence have higher mobility and metastatic capability (Prat et al. 2010; Sabatier et al. 2014).

Currently, the clinical value of molecular classification of breast cancer is limited to the correspondence between subgroups of breast tumors and the status of ER, PR and HER2, as well as tumor grades (Sotiriou and Pusztai 2009). However, several studies have suggested that it can provide better predictions of clinical outcome than the traditional classifications based on histopathological characters (Foekens et al. 2006; Naderi et al. 2006). Gene expression profiling may also help guide clinicians to select the best treatment options for breast cancer patients. For example, based on the expression of 70 genes, researchers from the Netherlands Cancer Institute have developed a genetic test, termed MammaPrint, which can predict prognosis (van't Veer et al. 2002). A comparison of this test with the Adjuvant! Online program (www.adjuvantonline.com), which uses conventional criteria, suggested that MammaPrint appeared to be more accurate in predicting prognosis of breast cancer patients (Drukker et al. 2013). Another molecular assay, Oncotype DX (Genomic Health), measures the expression of 21 genes, including ER, HER2 and ER-regulated genes with reverse transcription quantitative PCR (RT-qPCR). This assay was shown to be able to identify the ER-positive tumors that are more likely responsive to tamoxifen treatment (Habel et al. 2006).

1.1.3 Inherited breast cancer predisposition is often associated with DNA repair genes

Most breast cancers are caused by accumulation of somatic mutations in breast tissues, which are not heritable, so they are called sporadic breast cancer (Olopade et al. 2008). A small proportion (5–10%) of breast cancers, however, can be attributed to single mutations inherited from the patients' parents, so they are called hereditary breast cancer. Usually one mutant allele is inherited from either parent while the other allele remains wildtype, so the affected gene is heterozygous and can produce a functional protein (Staff et al. 2000). However, tissue-specific loss of heterozygosity (LOH) usually happens, which inactivates the wildtype allele and eventually causes breast cancer (Antoniou and Easton 2006).

The first breast cancer susceptibility gene was mapped to chromosome 17q21 by linkage studies of families with early-onset breast cancers, and it was named *BRCA1* (breast cancer 1, early onset) (Hall et al. 1990; Miki et al. 1994). It was followed by the discovery

of a second gene named *BRCA2* (breast cancer 2, early onset), which is located in chromosome 13q12–13 (Wooster et al. 1994). Following studies demonstrated that both BRCA1 and BRCA2 play very important roles in DNA damage response and repair. Therefore, mutations in either gene cause accumulation of damaged DNA, which leads to genomic instability and eventually cancer development (Venkitaraman 2002; King et al. 2003; Venkitaraman 2009). *BRCA1* and *BRCA2* are high-penetrance breast cancer susceptibility genes, and those women bearing one-allele mutations have up to 80% chances of developing breast cancer in their lifetime. Mutation of *BRCA1* or *BRCA2* also gives a woman up to 50% chance of ovarian cancer development in her lifetime (King et al. 2003).

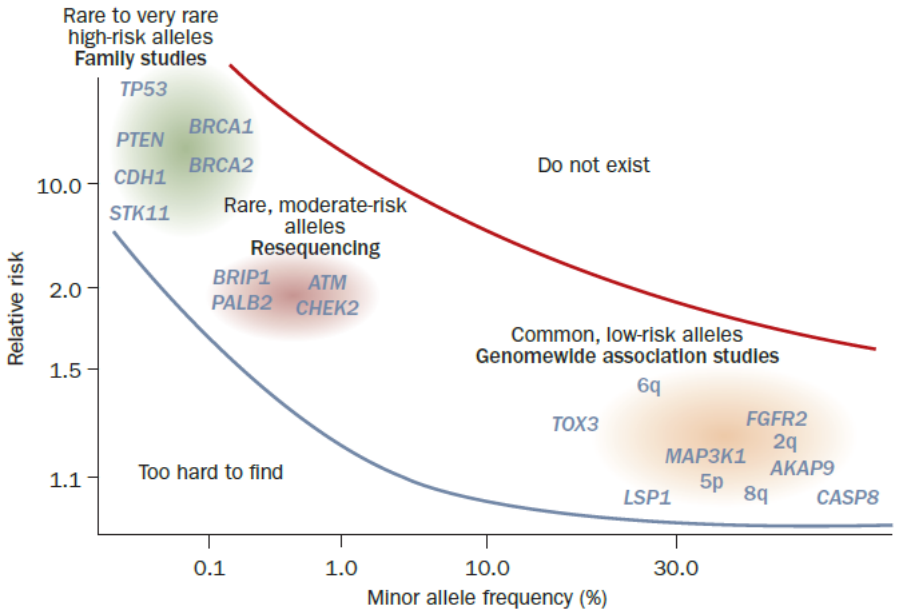

Figure 2. Relative risk factors of breast cancer susceptibility genes and their mutation frequencies among general population. (Reproduced from Harris and McCormick 2010, with the permission of reprints & permissions@npg)

Following the discoveries of *BRCA1* and *BRCA2*, several other breast cancer susceptibility genes with different risk factors have been found through large-scale or linkage studies (Figure 2) (Rahman et al. 2006; Meindl et al.2010; Easton et al. 2007). Interestingly, most of these genes have functions in a common pathway: DNA damage response and repair. Partner and localizer of BRCA2 (PALB2) mediates the interaction of BRCA1 and BRCA2, which is required for loading RAD51 recombinase during homologous recombination. Mutations of *PALB2* increase two- to three-fold risks of breast cancer development, which categorizes it as a medium-penetrance breast cancer susceptibility gene (Poumpouridou and Kroupis 2012). Several studies have shown that a truncating mutation of *CHEK2*, CHEK2*1100delC, increases two-fold risk of breast cancer

development. Additionally, this mutation multiplies the risks associated with mutations of other breast cancer susceptibility genes (CHEK2 Breast Cancer Case-Control Consortium 2004; Meijers-Heijboer et al. 2002). ATM, NBS1, RAD50 and BRIP1 are also involved in the regulation of DNA damage response and repair, and they are found to be low- to modest-penetrance breast cancer susceptibility genes (Walsh and King 2007). The tumor suppressor genes *TP53* and *PTEN* are mutated in a large number of sporadic cancers, including breast cancers. Germline mutations of these genes, although rare, confer high risks of breast cancer development as well (Garcia-Closas and Chanock 2008). Recently, several genome-wide association studies have identified additional breast cancer susceptibility loci, with the functions of several genes awaiting further studies (Easton et al. 2007; Turnbull et al. 2010).

1.2 DNA damage response and repair

1.2.1 Cancer cells often experience elevated DNA damage

Human cells are constantly challenged with DNA damage caused by both endogenous and environmental factors. Products released by metabolic processes, e.g. reactive oxygen species (ROS) and reactive nitrogen species (RNS) are common chemicals that damage DNA (Martin 2008). Chemical bonds of DNA can be cleaved directly through hydrolysis, which is another source of endogenous DNA damage (Gates 2009). Many environmental reagents can induce DNA damage, e.g. UV light from sunlight, ionizing radiation (IR) released by radioactive substances and various chemical compounds. Cells from human body have evolved sophisticated machineries to cope with different kinds of DNA damage, mostly by repairing them. If damaged DNA cannot be repaired due to various reasons, cells may undergo senescence or apoptosis (Freitas and de Magalhaes 2011). However, in many cases, some cells can survive DNA insults without repairing them properly. This may lead to accumulation of DNA damage, genomic instability and eventually initiation of cancer (Hoeijmakers 2009; Jackson and Bartek 2009). More than 90% of skin cancers are caused by exposure to sunlight, from which UV light induces certain DNA damage, mainly pyrimidine dimers (Hill et al. 2013). Smoking greatly increases the risks of lung cancer development, because tobacco contains many carcinogens that induce DNA damage (Pfeifer et al. 2002). Both of these examples indicate that elevated DNA damage contributes to the development of cancer.

Dysfunction of DNA damage repair is observed in a large number of cancers, which is another piece of evidence suggesting that those cancer cells have experienced elevated DNA damage (Jackson and Bartek 2009). 20–30% of hereditary breast cancers are caused by germline mutations of *BRCA1* or *BRCA2*, both of which are involved in the repair of DNA DSBs through homologous recombination pathway (Venkitaraman 2002; Antoniou et al. 2002). In addition, a subset of hereditary breast and ovarian cancers can be attributed

to mutations of other genes involved in homologous recombination pathway, e.g. *ATM*, *PALB2* and *RAD51C* (Walsh and King 2007). Defective mismatch repair system, resulting from mutations of *MLH1* or *MSH2*, causes microsatellite instability (MSI) and confers high risks of developing hereditary nonpolyposis colorectal cancer (HNPCC, also called Lynch syndrome) (Poulogiannis et al. 2010). Microsatellite instability is observed in 15–20% sporadic colon cancers, further indicating contribution of dysfunctional DNA damage repair to cancer development (Poulogiannis et al. 2010).

The theory of DNA damage-induced tumorigenesis was established based on a seminal model proposed by Halazonetis et al. (2008). In precancerous cells with functional p53, activated oncogenes induce stalling and collapse of DNA replication forks, which lead to DSBs at very low rates, as indicated by foci formation of 53BP1 and γH2AX (DiTullio et al. 2002; Gorgoulis et al. 2005). In these cells, functional DNA repair proteins, known as caretakers in comparison to classical tumor suppressors, can efficiently repair damaged DNA. Alternatively, these double-strand breaks can activate p53, which acts as a barrier of tumorigenesis by inducing apoptosis or senescence (Schultz et al. 2000; Di Micco et al. 2006). However, a few cells may bypass this barrier by losing the functions of p53, or less commonly, other tumor suppressors. This would result in the amplification of cells with damaged DNA, which leads to genomic instability and eventually cancer development. This model features two common characters of cancer: genomic instability resulted from the accumulation of DNA damage; and frequent mutations of *p53* and other tumor suppressor genes in cancer cells (Halazonetis et al. 2008). Considering the fact that DNA repair proteins play such important roles in preventing tumorigenesis by clearing DNA damage, it is conceivable that mutations of these genes would accelerate tumorigenesis. This is indeed the case with the caretaker genes, e.g. *BRCA1*, *BRCA2* and *ATM*, whose mutations confer high risks of breast cancer development (Walsh and King 2007).

1.2.2 Regulation of DNA damage response and repair

DNA damage repair is a complex process that is highly coordinated with cell cycle checkpoint regulation (Polo and Jackson 2011). In response to DNA damage, cell cycle checkpoints are activated to temporarily halt cell proliferation, which allows time for a cell to repair DNA lesions. Depending on the stages of cells upon DNA damage, they can be stopped at different points: before DNA replication (G1 checkpoint), during replication (intra-S checkpoint) or before cell division (G2/M checkpoint) (Polo and Jackson 2011; Smith et al. 2010). Selection of DNA repair pathways depends mainly on the types of DNA damage, with some pathways having overlapping functions in substrate recognition (Figure 3).

Direct repair: The simplest form of DNA repair is to directly reverse damage incurred to only one of the four bases. UV light induces pyrimidine dimers that are

recognized by repair enzymes in response to local conformational changes (Cooper 2000). The dimers can be repaired through photoreactivation, in which an enzyme called photolyase directly disrupts the dimers via photochemical reactions with the energy from visible light. Consequently, the dimerized bases are restored to their original forms. This repair mechanism, however, exists only in a few species, but not in human (Cooper 2000).

Another example of direct repair is removal of the methyl group from O^6-methylguanine by the enzyme O^6-methylguanine methyltransferase (MGMT) (Curtin 2012). Guanine can be methylated at O6 position upon exposure to alkylating agents, which results in mismatch or errors during replication or transcription. MGMT repairs one O^6-methylguanine at the cost of one enzyme, since it transfers the methyl group into its own cysteine residue, which results in the loss of its enzymatic activity (Pegg 1990).

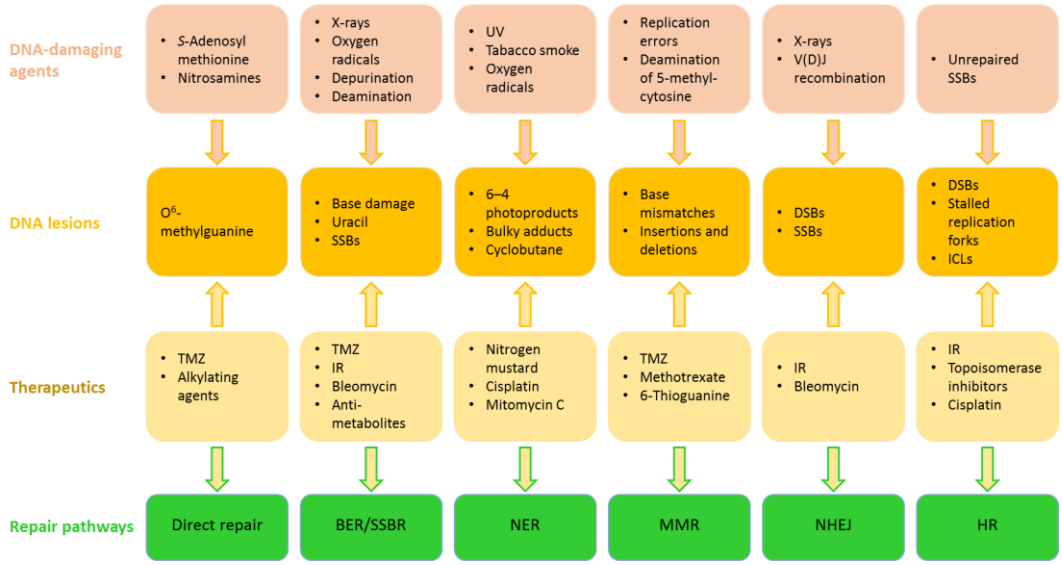

Figure 3. DNA lesions induced by different DNA-damaging agents or therapeutic drugs and their repair pathways. (Modified from Helleday et al. 2008; Curtin 2012; Lord and Ashworth 2012)

Nucleotide excision repair (NER): Since photolyase-mediated direct repair does not exist in human or other placental mammals, repair of pyrimidine dimers in these species relies on another pathway: nucleotide excision repair. Dysfunctional NER leads to several diseases, from which many of the NER components are named, e.g. xeroderma pigmentosum (XPA through XPG) and Cockayne syndrome (CSA and CSB) (Kraemer et al. 2007). There are two subpathways of NER: global genomic NER (GG-NER) and transcription-coupled NER (TC-NER), which differ in recognition of DNA lesions but share the same repair processes (Costa et al. 2003). GG-NER is functional in the whole genome during any cell stages. The damage sensing proteins, DNA damage binding 1 and 2 (DDB1 and DDB2) and XPC-HR23B complex, constantly scan the genome and bind to damaged or distorted sites of the DNA double helix (Gillet and Schärer 2006). In contrast

to GG-NER, TC-NER preferably repairs DNA lesions during transcription (Hoeijmakers 2009). It uses RNA polymerase stalled at DNA lesions as a sensing signal, which attracts and stabilizes CSA and CSB. After recognizing DNA lesions, GG-NER and TC-NER converge to the same processes for damage repair. Several NER repair factors are recruited to the damage sites, including transcription factor II Human (TFIIH), XPA, XPG and replication protein A (RPA) (Sugasawa et al. 2001). TFIIH is a multi-subunit complex containing XPB and XPD, which act as DNA-dependent ATPase and DNA-dependent helicase, respectively, to unwind the double helix around the lesion (Fousteri and Mullenders 2008). RPA helps to protect single-stranded DNA and it is also involved in several other repair pathways when single-stranded DNA is exposed (Sancar et al. 2004). Then a few nucleotides flanking the damaged base are removed by the endonucleases XPG on 3' side and the XPF-ERCC1 heterodimer on 5' side. The dual incision leads to a single-stranded gap of 25–30 nucleotides (O'Donovan et al. 1994; Sijbers et al. 1996). The single-stranded gap is then filled by DNA polymerases using the other undamaged strand as a template.

Base excision repair (BER): Base excision repair also repairs small DNA lesions involving one or few bases, but differs from NER in that the affected helix is non-distorting (Marteijn et al. 2014). Several types of DNA damage, e.g. oxidized bases, alkylated bases and deaminated bases are the targets of BER. Repair of DNA single-strand breaks (SSBs), termed single-strand break repair (SSBR), is generally regarded as a subpathway of BER (Almeida and Sobol 2007).

BER starts with recognition and excision of a damaged base by one of several glycosylases to form an AP site (apurinic/apyrimidinic site, abasic site) (Almeida and Sobol 2007). Monofunctional glycosylases can only remove damaged nitrogenous base by cleaving the N-glycosidic bond and leave the sugar-phosphate in the DNA backbone. Bifunctional glycosylases also possess AP lyase activity that allows them to remove the AP site, which produces a single-strand break (Jacobs and Schär 2012). Examples of glycosylases are monofunctional uracil-DNA glycosylase (UNG), which removes inappropriately incorporated or deaminated uracil; and bifunctional 8-oxoguanine DNA glycosylase (OGG1), which removes 8-oxoguanine (8-oxoG) (Jacobs and Schär 2012). Recognition of DNA damage is facilitated by PARP1 and PARP2 (Schreiber et al. 2002; Wang et al. 2006). Consequently, inhibition of PARP leads to the accumulation of single-strand breaks, which are converted to double-strand breaks when they encounter replication forks. Binding of PARP1 to DNA lesions not only activates its enzymatic activity, but also is required for the recruitment of X-ray repair cross complementing 1 (XRCC1), another key component of BER (Brem and Hall 2005). The AP site created by monofunctional glycosylases is cleaved by the AP endonuclease to produce a single-strand break, which is

then processed by polynucleotide kinase 3'-phosphatase (PNKP) to form termini suitable for ligation: 3' hydroxyl and 5' phosphate (Caldecott 2008). The single-stranded gap is filled by DNA polymerase β or λ and finally sealed by DNA Ligase III and its cofactor XRCC1 (Braithwaite et al. 2005).

Mismatch repair (MMR): During DNA replication and recombination, insertion, deletion and misincorporation of nucleotides happen frequently in the newly synthesized strand. The evolutionarily conserved mismatch repair system is responsible for correcting these errors. In eukaryotes, a mismatch is recognized by either MSH2-MSH6 (MutSα) or MSH2-MSH3 (MutSβ) heterodimer, the homologs of *E. coli* protein MutS (Kunkel and Erie 2005). Studies have shown that MutSα preferably binds to single base-base or insertion-deletion mismatches (IDLs), while MutSβ is mainly responsible for larger IDLs, although the two heterodimers share certain substrates, especially single IDLs (Kunkel and Erie 2005; Harrington and Kolodner 2007). Activities of MutSα and MutSβ are facilitated by MutLα complex, which is composed of MLH1 and PMS2 (Li 2007). MutLα possesses endonuclease activity, which creates strand breaks with the help of MutSα and PCNA/RFC (Kadyrov et al. 2006). PCNA also participates in DNA resynthesis after strand excision, which is elongated by Polδ and sealed by DNA Ligase I (Longley et al. 1997; Zhang et al. 2005).

Loss of MMR activity is associated with several cellular defects, most profoundly increased microsatellite instability (MSI) (Thibodeau et al. 1996). MSI is a result of frequent DNA polymerase slippage and inefficient DNA proofreading (Eckert and Hile 2009). It has been established as a biomarker of MMR deficiency in cancer cells. For example, most of the hereditary nonpolyposis colorectal cancers (HNPCCs) are caused by mutations of mismatch repair genes *MLH1* and *MSH2*, and they are enriched for MSI (Poulogiannis et al. 2010). Unlike many other DNA repair pathways, loss of which sensitizes cells to DNA damage, defective MMR causes resistance to DNA-damaging agents (Curtin 2012). For example, the human lymphoblastoid cell line MT1 is resistant to the alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) because of defective MSH6, another component of the mismatch repair pathway (Kat et al. 1993). XPA-deficient cells are usually more sensitive to UV light due to loss of NER. When a new mouse skin cancer cell line was established by UVB irradiation of Xpa-deficient mice, these cells showed resistance to UV light, 6-thioguanine and had defective cell cycle checkpoints (Ichikawa et al. 2000). Further studies demonstrated that the resistant phenotypes were attributed to lower expression of several MMR proteins: Msh2, Msh3 and Msh6.

Non-homologous end joining (NHEJ): DNA double-strand breaks are the most deleterious damage in a cell, and are among the most difficult lesions to repair. They can

be induced exogenously by radiation or radiomimetic drugs. They also happen spontaneously during normal cellular processes, including replication, meiosis and V(D)J recombination (Pfeiffer et al. 2000). DSBs are mainly repaired by two mutually exclusive pathways: homologous recombination (HR) or non-homologous end joining (NHEJ) (Pfeiffer et al. 2000). HR requires the sister chromatid as a template to resynthesize missing nucleotides, so it is error free but can only happen in S and G2 phases; while the error-prone NHEJ does not need any template, and hence it can repair DSBs throughout the whole cell cycle (Mahaney et al. 2009; Shrivastav et al. 2007). There is also an alternative NHEJ called microhomology-mediated end joining (MMEJ), which involves a short resection of the broken ends (McVey and Lee 2008).

Despite the fact that HR is a more accurate repair mechanism, studies have shown that NHEJ is a predominant pathway for the repair of DSBs, even during S and G2 phases when a sister chromatid is available (Beucher et al. 2009). This is mainly due to the abundance of Ku proteins, which have a strong affinity to DNA ends of DSBs (Mahaney et al. 2009). After binding to double-strand break ends, Ku70/Ku80 heterodimer recruits and activates the catalytic subunit of DNA-dependent protein kinase, DNA-PKcs, which in turn stabilizes the broken ends and prevents them from being resected (Meek et al. 2008). Full activation of DNA-PKcs requires a series of autophosphorylation at several serine and threonine residues, which releases the broken ends to allow NHEJ to proceed. DNA-PKcs also phosphorylates other substrates involved in NHEJ, including RPA, WRN and Artemis (Collis et al. 2004; Cui et al. 2005; Goodarzi et al. 2006). In addition, activated DNA-PKcs stimulates juxtaposition of the broken DNA ends for re-joining (Jovanovic and Dynan 2006). DNA ends of DSBs usually need to be processed so that they are compatible for re-joining. A few proteins are responsible for this process: Artemis, PNKP, DNA polymerases, and the MRN (MRE11, RAD50 and NBS1) complex (Drouet et al. 2006). Artemis protein possesses endonuclease activity, which is required to open the hairpin end together with DNA-PK during V(D)J recombination (Ma et al. 2002). Ku heterodimer also recruits polymerases μ and λ via their BRCT domains to the DSB sites, where they fill the gap between two broken ends (Ma et al. 2004). Finally, XLF-stimulated XRCC4/DNA Ligase IV complex is recruited to seal the gap (Yano et al. 2008).

Microhomology-mediated end joining (MMEJ): In some circumstances when the classical NHEJ is not available, cells rely on an alternative NHEJ, microhomology-mediated end joining, to repair DSBs (McVey and Lee 2008). MMEJ is initiated by PARP1, which competes with Ku proteins for binding to DSB ends. Studies have shown that when there is a short homology between two broken ends, not all the NHEJ components are needed to repair the damage (Weinstock et al. 2007; Jung et al. 2006). Similar to homologous recombination, MMEJ needs some end resection, but for a much shorter

sequence (Truong et al. 2013). After aligning of the short (micro) homologs, any overhung nucleotide from the broken ends is chopped away, and the paired ends are ligated by XRCC4/Ligase IV complex. Since MMEJ deletes nucleotides flanking the broken sites, it always introduces changes in DNA sequence, which might contribute to the oncogenic chromosome rearrangements (McVey and Lee 2008).

Homologous recombination (HR): Repair of DNA double-strand breaks by homologous recombination is a multi-step process that requires a large protein complex and dynamic chromatin changes. DSBs are sensed by the MRN complex, which also plays important roles in the regulation of cell cycle checkpoints and DNA end resection (Yuan and Chen 2010). Binding of the MRN complex to DSB sites stabilizes the broken ends, where it recruits and promotes the activation of ATM to elicit CHK2-mediated cell cycle checkpoints (Bruhn et al. 2014). Activated ATM also induces rapid phosphorylation of histone H2AX at Ser139 (γH2AX), which serves as a signal to recruit a number of repair factors at the DNA damage sites (Bonner et al. 2008). Mediator of DNA damage checkpoint 1 (MDC1) is one of the earliest factors to be recruited, which directly binds to phosphorylated H2AX through its tandem BRCT domains (Stewart et al. 2003). γH2AX-bound MDC1 is constitutively phosphorylated by casein kinase 2 (CK2), which promotes its interaction with NBS1 and thus retention of the MRN complex at the DNA damage sites, where it fully activates ATM and further stimulates ATM-mediated phosphorylation of H2AX and other substrates (Chapman and Jackson 2008; Spycher et al. 2008). The loop between H2AX phosphorylation and MRN recruitment was best explained by the study which demonstrated that initial recruitment of MRN complex, as well as 53BP1 and BRCA1 is independent of γH2AX (Celeste et al. 2003). Rather, H2AX phosphorylation is required for the amplification of DNA damage signaling cascade to recruit large amount of proteins for DSB repair (Bonner et al. 2008).

A crucial step that determines the initiation of HR is resection of the DSB ends, which generates 3' single-stranded DNA (ssDNA) (Figure 4). It is highly regulated by multiple factors, including the MRN complex (Petrini and Stracker 2011; Lavin 2004), CtBP-interacting protein (CtIP) (Sartori et al. 2007), DNA replication helicase/nuclease 2 (DNA2) and EXO1 (Symington and Gautier 2011). MER11 of the MRN complex possesses nuclease activity, which is promoted by CtIP (Chen et al. 2008). Studies in *S. cerevisiae* showed that the MRE11-RAD50-XRS (MRX, homologue of MRN in human) complex and SAE2 (homologue of CtIP in human) are responsible for the initial limited processing of the 5' strand (Mimitou and Symington 2008). In humans, the role of CtIP in DNA end resection was first demonstrated by Alessandro Sartori et al. (Sartori et al. 2007), whose work showed that CtIP physically interacts with the MRN complex and is required for the recruitment of downstream factors. Further studies detailed that CtIP is not only

required for repair of DSBs by HR in S/G2 phases, but also for MMEJ in G1 phase, both of which involve DNA end resection (Yun and Hiom 2009). DSB DNA ends can be also unwound by a RecQ-type DNA helicase (Sgs1, BLM or WRN), and one of the ssDNA is degraded by DNA2, which has 5′→3′ nuclease activity (Gravel et al. 2008; Liao et al. 2008). In contrast, EXO1 can use its 5′→3′ exonuclease activity to directly degrade DSB DNA ends (Liao et al. 2011).

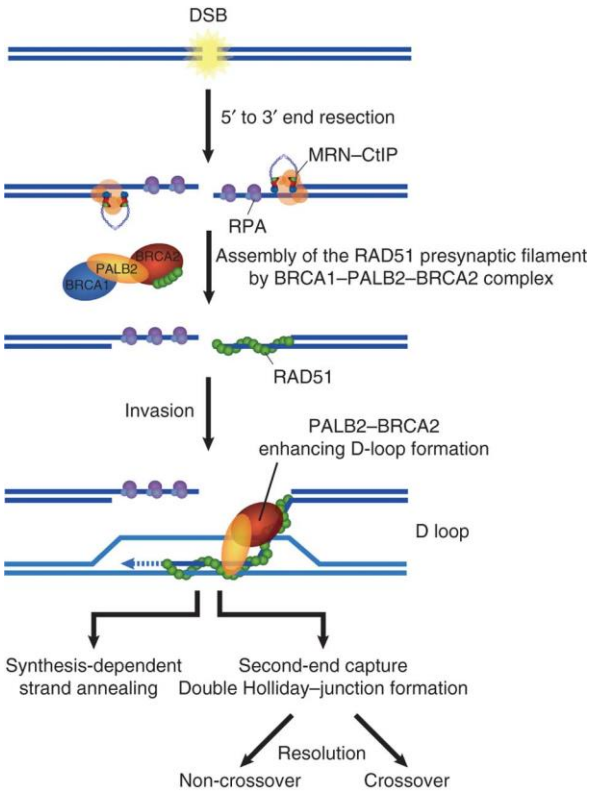

Figure 4. A model of DSB repair through homologous recombination pathway, which is initiated with the resection of DSB ends to expose 3' overhangs that are coated by RPA. Together with BRCA2 and PALB2, BRCA1 helps to load RAD51 recombinase into the single-stranded DNA. (Reproduced from Buisson et al. 2010, with the permission of reprints & permissions@npg)

After DNA end resection, the 3' single-stranded DNA is coated with replication protein A (RPA), which prevents formation of DNA hairpins (Chen et al. 2013). RAD51, a key recombinase required for homologous recombination, is recruited to the DSB sites through interaction with BRCA1 and BRCA2, where it displaces RPA and forms a RAD51-ssDNA nucleofilament (Yu et al. 2001; Roy et al. 2011). The RAD51-coated ssDNA then starts to search for a homologous sequence, after which RAD51 catalyses the invasion and exchange of the homologous sequences to form a D-loop structure (Figure 4) (Bianco et al. 1998). The D loop can be unwound and the freed ssDNA strand anneals with the strand associated with the other DSB end (San Filippo et al. 2008). This reaction is completed by

gap-filling DNA synthesis and re-ligation, and it produces only noncrossover sequences. The D loop may also form two Holliday junctions after the second DSB end is captured by the invaded ssDNA, accompanied by gap-filling DNA synthesis and re-ligation (San Filippo et al. 2008). DNA Polδ is the main polymerase responsible for DNA resynthesis with the help of PCNA and PIF1 helicase (Maloisel et al. 2008; Li et al. 2009; Wilson et al. 2013). The two Holliday junctions are then resolved by the SLX-MUS complex (SLX1-SLX4 and MUS81-EME1) to form crossover or noncrossover sequences (Wyatt et al. 2013); alternatively, Holliday junctions can be dissolved by the BTR complex (BLM helicase, Topoisomerase IIIα, RMI1 and RMI2) to form exclusively noncrossover sequences (Wu and Hickson 2003).

Because HR needs a sister chromatid as a template to synthesize depleted nucleotides during strand extension, it can only happen in S and G2 phases (Shrivastav et al. 2007). For the same reason, efficient HR repair keeps genomic information intact and helps to sustain genomic integrity. Choice of HR repair is determined by several factors besides cell cycle stages (Shrivastav et al. 2007; Symington and Gautier 2011). For example, 53BP1 has emerged as an important protein in response and repair of DSBs, which favours NHEJ but supresses HR (Kakarougkas et al. 2013). As binding of Ku70/80 to DSB ends initiates NHEJ, it is presumable that Ku proteins need to be kept away for HR initiation (Shao et al. 2012). Studies in yeast have shown that the MRN complex, which plays key roles in HR repair, prevents Ku proteins from binding to the broken DNA ends (Symington and Gautier 2011). Similar to its role in MMEJ, PARP1 also competes with Ku to bind DSB ends and facilitates end resection, which is one of the essential steps of HR (Hochegger et al. 2006).

1.2.3 Targeting DNA damage repair pathways for the treatment of cancer

DNA damage not only is the cause of genomic instability and cancer development, but also can be exploited for the treatment of cancer. In contrast to malignant cells, which are usually defective for certain DNA repair pathways, normal cells in a human body express functional DNA repair proteins and thus are able to survive certain types of DNA damage. This fundamental difference has been extensively exploited for the development of cancer therapeutics (Dietlein et al. 2014). In-depth studies of DNA damage response and repair would help to better understand the molecular mechanisms of drug resistance, and guide us to design more effective therapeutic drugs.

The principle of DNA damage-based chemotherapy was discovered accidentally during World War I, when a group of people exposed to mustard gas were found to have low levels of the rapidly dividing white blood cells (DeVita and Chu 2008). Later on, a few patients with advanced lymphomas received the treatment of mustard gas intravenously, which resulted in remarkable improvements (Gilman 1963). Further studies demonstrated that mustard gas can induce DNA damage by alkylating the guanine nucleotide, which

leads to apoptotic cell death if not repaired properly (Kehe et al. 2008). Since then, various chemotherapeutics inducing different types of DNA damage have been developed for the treatment of cancer (Figure 3).

Alkylating agents: Alkylating agents induce DNA damage by adding their alkyl groups into DNA helix, which results in bulky adducts, interstrand or intrastrand crosslinks (Fu et al. 2012). Alkylating agents can be classified into several groups: nitrogen mustards, nitrosoureas, alkyl sulfonates, triazines and ethylenimines (Brulikova et al. 2012). The nitrogen mustard group members, mechlorethamine, cyclophosphamide and melphalan, attach to the N7 position of guanines and induce interstrand crosslinks (ICLs), which usually need to be repaired by NER or HR pathway (Povirk and Shuker 1994; Noll et al. 2006). Nitrogen mustards are widely used in treating lymphomas, lung and breast cancers among other malignant diseases. The triazine family member temozolomide (TMZ) has been used for the treatment of glioblastoma and melanoma (Jiang et al. 2011). TMZ methylates the N7 or O6 position of a guanine base, which can be removed by O^6-methylguanine methyltransferase (MGMT) (Jiang et al. 2011). As a result, loss of MGMT activity sensitizes cells to TMZ. TMZ can be administrated with the pseudo-substrate of MGMT, O^6-benzylguanine (O6-BG), which has been shown to substantially increase the sensitivity of tumor cells to TMZ (Dolan et al. 1990). Moreover, expression of MGMT is often silenced in glioblastoma due to methylation of its promoter (Hegi et al. 2005), which sensitizes cells to TMZ. On the other hand, tumors expressing high levels of MGMT are predicted to be resistant to TMZ.

The platinum-based chemotherapeutic drugs, including cisplatin, carboplatin and oxaliplatin, are usually classified as alkylating-like agents, because they also induce DNA crosslinks and require HR and NER for repair (Brulikova et al. 2012). Cisplatin was the first of its kind to be developed and has been used for the treatment of many kinds of malignancies, including testicular, ovarian, cervical, head and neck, and small-cell lung cancers (Luo et al. 2012). It mainly induces intrastrand crosslinks by covalently bonding the N7 positions of adjacent guanines, which need to be repaired by the NER system (Noll et al. 2006). As a result, cells with defective NER, e.g. xeroderma pigmentosum (XP) cells, are especially sensitive to cisplatin (Cleare et al. 1980). Several studies have shown that overexpression of XPA and ERCC1, another protein involved in NER, correlates with cisplatin resistance, as these cells can efficiently repair DNA damage induced by cisplatin (Stevens et al. 2005; Steffensen et al. 2008). Platinum agents also induce ICLs that require HR pathway for repair. In addition, processing of cisplatin-induced crosslinks leads to double-strand breaks in replicating cells (Kondo et al. 2010). Hence, cancer cells with defective DSB repair systems, e.g. BRCA1/2-mutant cells, are predicted to be more sensitive to platinum drugs.

Antimetabolites: Antimetabolites are nucleobase or nucleotide analogues that interfere with DNA or RNA synthesis. They can either inhibit the enzymes required for DNA synthesis or incorporate into DNA or RNA, which prevents their synthesis and induces cell death (Kinsella et al. 1997). The exact repair mechanisms responsible for antimetabolite-induced DNA damage are still unclear, but BER/SSBR is involved in several processes (Helleday et al. 2008). For example, the folate antimetabolites fluorouracil and raltitrexed inhibit thymidylate synthase, which results in deprivation of thymidine and causes DNA single-strand breaks that activate BER/SSBR pathway (Seiple et al. 2006; Longley et al. 2003). Raltitrexed has been approved for the treatment of colorectal cancer, while fluorouracil is widely used in treating anal, breast, colorectal, stomach, pancreatic and some other cancers (Kaye 1998).

Topoisomerase inhibitors: During replication and transcription, supercoiled DNA needs to be unwound for the access of polymerases and other proteins. This process is facilitated by two enzymes: type I topoisomerase (Top 1) and type II topoisomerase (Top 2) (Wang 2002). Inhibition of Top1 or Top2 induces SSBs and DSBs, which need to be repaired by SSBR and NHEJ/HR, respectively (Helleday et al. 2008). The topoisomerase I inhibitors irinotecan and topotecan are derived from the natural alkaloid camptothecin. They trap Top1 cleavage complexes produced during relaxation and re-ligation of DNA double strands, leading to SSBs and DSBs (Plo et al. 2003). Etoposide (VP-16), anthracyclines (doxorubicin, daunorubicin) and mitoxantrone are widely used Top2 inhibitors (Nitiss 2009). Etoposide forms ternary complex with DNA and Top2 enzyme, which prevents re-ligation of DNA strands and leads to strand breaks. Doxorubicin and daunorubicin can intercalate into DNA, which inhibits the process of Top2 during transcription. They can also stabilize Top2 during DNA replication, thus leading to DNA strand breaks. Doxorubicin is widely used in combination chemotherapy for the treatment of various cancers, for example: AC (doxorubicin, cyclophosphamide) and CMF (cyclophosphamide, methotrexate and 5-fluorouracil) in breast cancer; and ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) in Hodgkin's lymphoma (Fiets et al. 2003; Santoro et al. 1987).

Radiation and radiomimetic agents: Radiation and radiomimetic agents induce double-strand breaks directly, which need to be repaired by NHEJ or HR pathway (Helleday et al. 2008). Additionally, radiation can also induce ROS, which causes other types of DNA damage (Yamamori et al. 2012). Radiotherapy is considered as local treatment, since the radiation beams are directed towards the cancerous tissues, which provides a much higher absorbed dose to cancer cells than to the surrounding normal cells. In addition to adjuvant and neoadjuvant therapies, radiation can be used as a curative therapy with the aim of curing cancer without surgery (Delaney et al. 2005). It is commonly

used in combination with chemotherapies for the treatment of various types of cancer. However, different cancers may have different sensitivities to radiotherapy. Some cancers can be easily killed by a modest dose of radiation, e.g. leukemia, lymphoma and germ cell tumors. In contrast, most epithelial tumors require much higher dose of radiation for cure. The radiomimetic drug bleomycin is often used with other anticancer drugs for the treatment of several cancers. For example, it is used in combination with etoposide and cisplatin (BEP) in testicular cancer; with doxorubicin in Hodgkin's lymphoma (Lewis and Nydorf 2006).

Targeting cell cycle checkpoints: DNA damage response involves temporary halt of the cell cycle progression to give cells time for DNA repair. Without proper cell cycle control, cells would divide with massive DNA damage, which cannot be repaired but leads to cell death (Bouwman and Jonkers 2012). The PI3K-related kinase (PIKK) family members, ATM, ATR and DNA-PK, play central roles in DNA damage response by activating cell cycle checkpoints (Bouwman and Jonkers 2012; Falck et al. 2005). Consequently, inhibition of these kinases abolishes checkpoint activation induced by DNA-damaging agents, which would lead to continuous cell division with massive DNA damage. Hence, combination of checkpoint kinase inhibitors and DNA-damaging agents provides a plausible strategy for cancer treatment. In preclinical studies, it has been shown that KU-55933, a small molecule ATM inhibitor, sensitizes cancer cells to ionizing radiation and several other DSB-inducing agents (Hickson et al. 2004). NU7441, a highly potent and selective DNA-PK inhibitor, was found to sensitize cancer cells to both IR and etoposide (Zhao et al. 2006). However, due to the complex network pathways of ATM, ATR and DNA-PK, and their large number of substrates, inhibition of any of these kinases would result in unpredictable consequences. As a result, there is no inhibitor of these kinases reaching clinics at this stage (Hosoya and Miyagawa 2014).

Because loss of G1 checkpoint is a common feature in cancer cells, it is a reasonable strategy to target S and G2 phases for cancer treatment (Smith et al. 2010). The Thr/Ser kinases CHK1 and CHK2 are among the most important mediators of DNA damage response downstream of ATM, ATR and DNA-PK (Bouwman and Jonkers 2012; Smith et al. 2010). Several preclinical studies have raised the potential of targeting CHK1 or CHK2 for the treatment of cancer. For example, it has been demonstrated that inhibition of CHK1 in p53-mutant cancer cells abrogates G2 arrest caused by IR or cisplatin, thus sensitizing the cells to these agents (Carrassa et al. 2004). Several CHK1 and dual CHK1/CHK2 inhibitors in combination with various therapies are currently in clinical trials for treating different cancers (Hosoya and Miyagawa 2014).

Synthetic lethality: Synthetic lethality is a concept which means that concomitant inhibition of two molecules results in cell death, while inhibition of either one does not

change the viability, despite certain functional changes (Kaelin 2005). The phenomenon of synthetic lethality indicates the complexity of the biological systems. In cancer treatment, the concept was explored based on the fact that many cancer cells are defective for certain DNA repair pathways. As a result, these cells rely more on other repair pathways in order to survive (Kaelin 2005). During studies on developing effective therapeutics for the treatment of breast cancers associated with BRCA1/2 mutations, a breakthrough came when two groups independently demonstrated that inhibition of PARP1 selectively kills BRCA1- or BRCA2-deficient cells (Farmer et al. 2005; Bryant et al. 2005). Under normal conditions, PARP1 is involved in BER/SSBR pathway. Consequently, inhibition of PARP1 results in SSBs, which are converted to DSBs when they meet the replication forks. BRCA1/2-mutant cells cannot repair DSBs due to defective homologous recombination, in contrast to normal cells that have wildtype BRCA1/2 and thus functional HR. Consequently, BRCA1/2-mutant cancer cells die as a result of massive DNA damage, while normal cells survive after repairing damaged DNA. These findings quickly led to clinical trials of several PARP inhibitors (Audeh et al. 2009; Fong et al. 2009; Rouleau et al. 2010). Following a successful clinical trial, one of the PARP inhibitors, olaparib, was approved by FDA for the treatment of ovarian cancers associated with BRCA1/2 mutations. In addition, other preclinical studies have shown that PARP inhibitors also sensitize cancer cells mutant for other HR components (McCabe et al. 2006; Johnson et al. 2011; Turner et al. 2008), which raises a potential application of PARP inhibitors for the treatment of a wider spectrum of cancer patients.

1.3 BRCA1 as a cancer susceptibility gene

1.3.1 Functional domains of BRCA1 protein

Since the discovery of *BRCA1* as a high-penetrance breast and ovarian cancer susceptibility gene, much effort has been focused on deciphering its functions in the cells. Identification of several conserved domains in BRCA1 protein has helped to understand those functions (Figure 5) (Miki et al. 1994; Koonin et al. 1996; Sy et al. 2009). BRCA1 contains a highly conserved RING (really interesting new gene) finger domain in the N-terminus, and tandem BRCT (BRCA1 C-terminal) domains near the C-terminal end. Other important domains include two nuclear localization sequences (NLS) and a coiled-coil domain (Thakur et al. 1997; Sy et al. 2009). RING finger domains are usually found in E3 ubiquitin ligases, suggesting an E3 ligase activity of BRCA1 (Miki et al. 1994; Lipkowitz and Weissman 2011). BRCT domain is a phospho-protein binding motif and is usually found in proteins involved in cell cycle checkpoint control in response to DNA damage (Manke et al. 2003; Yu et al. 2003). The coiled-coil domain mediates the interaction of BRCA1 with PALB2, which is dependent on the phosphorylation of BRCA1 at Ser988 by CHK2 (Sy et al. 2009; Roy et al. 2011). PALB2 further interacts with BRCA2 and thus mediates the connection

between BRCA1 and BRCA2. An S/T-Q cluster domain (SCD) localizes after the coiled-coil domain, which contains several Ser/Thr sites that are phosphorylated by ATM in response to DNA damage (Cortez et al. 1999; Huen et al. 2010).

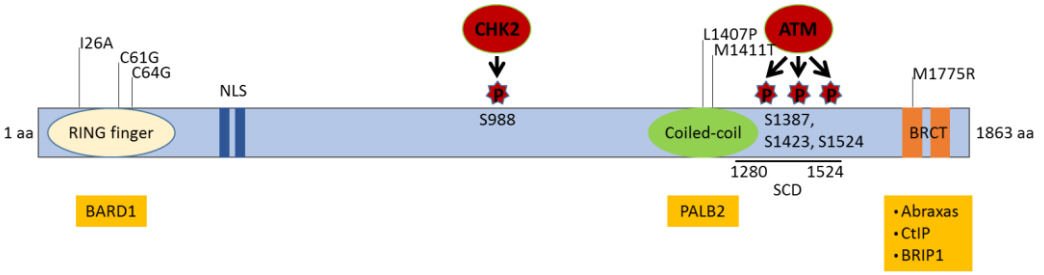

Figure 5. A scheme to show the main functional domains of BRCA1. Proteins interacting with BRCA1 during the processes of DNA damage response and repair are shown at the bottom. Those mutations found in clinics or routinely used in functional studies, e.g. C61G, C64G and I26A, are shown on the top. (Modified from Narod and Foulkes 2004; Huen et al. 2010; Roy et al. 2012)

The main function of BRCA1 has been found to be involved in DNA damage response and repair, where BRCA1 is required for the repair of DSBs through the error-free HR pathway (Venkitaraman 2002; Roy et al. 2011). Current data suggest that BRCA1 is involved in several steps of HR pathway (Table 1). First, through its E3 ligase activity, BRCA1 plays an important role in the amplification of DNA damage signals (Wu-Baer 2003; Huen et al. 2010). Second, several studies suggest that BRCA1 may facilitate DNA end resection, a key step that controls the initiation of homologous recombination (Cruz-Garc á et al. 2014). Third, BRCA1 is required for loading RAD51 recombinase, which is mediated by the interaction with PALB2 and BRCA2 (Xia et al. 2006). Finally, BRCA1 mediates the activation of cell cycle checkpoints to temporarily halt cell cycle progression in response to DNA damage (Deng 2006; Yarden et al. 2002). In addition to DNA damage response and repair, BRCA1 plays important roles in regulating mammary gland development and cell stemness, which may determine the unique phenotypes of breast tumors caused by its mutations (Smart et al. 2010; Liu et al. 2008).

1.3.2 BRCA1 is an E3 ubiquitin ligase

When BRCA1 was found to be a strong candidate of breast cancer susceptibility gene, sequence analysis revealed the existence of a RING finger domain at the N-terminus, suggesting that BRCA1 might possess E3 ubiquitin ligase activity (Miki et al. 1994). Soon after, Wu et al. (1996) identified another RING finger domain-containing protein that interacted with BRCA1, and it was named BRCA1-associated RING domain protein 1 (BARD1). They also found that two BRCA1 missense mutations, C61G and C64G, which compromise its E3 ligase activity, disrupted its interaction with BARD1. These missense mutations of BRCA1 confer high risks of breast and ovarian cancer development,

indicating that interaction of BRCA1 and BARD1 is required for its tumor suppressor function (Wu et al. 1996; Couch et al. 1996). Indeed, BRCA1 and BARD1 form a constitutive heterodimer *in vivo*, which is necessary for the stabilization of both proteins (Baer and Ludwig 2002; Joukov et al. 2001). Furthermore, BRCA1 E3 ligase activity is significantly enhanced after binding with BARD1, which catalyzes Lys6-, Lys48- and Lys63-linked ubiquitination (Nishikawa et al. 2004; Xia et al. 2003). The essential role of BARD1 in maintaining BRCA1 functions was further demonstrated by the finding that missense mutations of BARD1 that disrupt its interaction with BRCA1 are present in some hereditary breast and ovarian cancers (Ratajska et al. 2012). In addition, mouse models with mammary epithelial cell-targeted depletion of *Brca1*, *Bard1*, or both, have strikingly similar phenotypes in breast cancer development (Shakya et al. 2008).

Despite solid studies describing the involvement of BRCA1 in DNA damage response and repair, the exact role of BRCA1 as an E3 ubiquitin ligase in these processes remains poorly characterized. Early studies suggested that BRCA1 is required for the accumulation of ubiquitin conjugates at DNA damage sites (Morris and Solomon 2004; Polanowska et al. 2006). BRCA1 ubiquitinates several targets in response to DNA damage in a non-canonical manner, which signals functional modifications rather than proteasomal degradation. BRCA1 mediates ubiquitination of H2A type histones (H2A and H2AX), which is required to modulate DNA damage response signaling and chromatin dynamics (Chen et al. 2002). Besides BRCA1, other E3 ubiquitin ligases, including RNF168, TIP60-UBC13 and RNF2-BMI1 complexes, also mediate the ubiquitination of H2A histones (Mattiroli et al. 2012; Pan et al. 2011; Ikura et al. 2007). Additionally, in response to DNA damage, BRCA1 catalyzes the ubiquitination of its phosphorylation-dependent binding partner, CtIP, in a non-canonical manner (Yu et al. 2006). Ubiquitination of CtIP is required for its association with chromatin and regulation of G2/M checkpoint control following DNA damage (Yu et al. 2006). Moreover, the BRCA1/BARD1 heterodimer can autoubiquitinate, and the ubiquitinated complex has an increased affinity for binding to other DNA damage repair factors (Simons et al. 2006). Current data from *in vitro* studies suggest that the BRCA1/BARD1 E3 ligase can ubiquitinate several different proteins in a non-canonical manner, including FANCD2, p53 and RNA polymerase II (Vandenberg et al. 2003; Dong et al. 2003; Starita et al. 2005). However, the functions of BRCA1 in mediating ubiquitination of these substrates await further clarification.

In addition to DNA damage response, the E3 ligase activity of BRCA1 is involved in the regulation of other cellular processes. A study by Starita et al. (2004) showed that mono-ubiquitination of γ-tubulin by BRCA1/BARD1 heterodimer at lysines 48 and 344 is required for the regulation of centrosome numbers. Another study suggested that estrogen receptor α (ERα) can be monoubiquinated by BRCA1/BARD1 heterodimer at Lys302

(Eakin et al. 2007), which is abrogated by the cancer-predisposing mutations of BRCA1, C61G and C641G. Since BRCA1 mutations lead to cancer development primary in breast and ovarian tissues, this study reveals a potential link between BRCA1 mutation and tissue-specific carcinogenesis.

1.3.3 Functions of BRCA1 in DNA damage response and repair

The first evidence suggesting that BRCA1 might be involved in DNA damage repair came from the observation that BRCA1 formed nuclear dots in S-phase MCF-7 cells (Scully et al. 1997a). Importantly, BRCA1 dots in S-phase cells colocalized with RAD51, a protein that was already known to mediate strand exchange during recombination and DNA damage repair (Sung and Robberson 1995; Baumann et al. 1996). Moreover, localization of BRCA1 is similar to that of ATR, another protein known to contribute to DNA damage response (Scully et al. 1997a; Keegan et al. 1996). Therefore, it was conceivable that BRCA1 might be involved in DNA damage repair, together with RAD51. Considering previous studies, which showed that BRCA1 is required for early embryonic proliferation and development, and that its expression is highly increased in replicating cells (Lane et al. 1995), these data implied that BRCA1 might function with RAD51 in maintaining genomic integrity. Later on, it was shown that BRCA1 subnuclear localization changes dynamically following DNA damage caused by HU, UV, mitomycin C and IR, direct evidence of BRCA1 participating in DNA damage response (Scully et al. 1997b).

BRCA1-mediated DNA repair is carried out at the DNA damage sites containing multiple responsive proteins that form microscopically visible sub-nuclear structures, termed IR-induced foci (IRIF) (Chapman et al. 2012). Following DSBs, the MRN complex acts as a DNA damage sensor, which recruits and activates ATM at the DNA damage sites, where it induces rapid phosphorylation of histone H2AX at Ser139 (γH2AX) (Burma et al. 2001). H2AX is a member of the histone H2A family, and accounts for about 10% of total H2A (Bonner et al. 2008). Studies have shown that a single DSB induces H2AX phosphorylation within a region of as long as 30 kb around the broken site, reflecting dynamic chromatin remodelling following DNA damage induction (Shroff et al. 2004). Phosphorylation of H2AX is required for the amplification of DNA damage signaling cascade to recruit a large number of DNA repair factors, which also involves ubiquitination of various proteins (Bonner et al. 2008). MDC1 binds to γH2AX through its tandem BRCT domains, and it is phosphorylated by the chromatin-bound ATM, which leads to the phosphorylation-dependent recruitment of ubiquitin E3 ligase RNF8 (Kolas et al. 2007; Huen et al. 2007). Together with the E2 ubiquitin-conjugating enzyme 13 (UBC13), RNF8 catalyzes the initial Lys63-linked ubiquitination of γH2AX and possibly other proteins at the DNA damage sites. The ubiquitination signal is amplified by another E3 ligase, RNF168, also with the help of UBC13 (Doil et al. 2009). Ubiquitin conjugates at the DSB

sites are recognized by the ubiquitin-interacting motif (UIM) of receptor-associated protein 80 (RAP80), which recruits BRCA1 to the sites of DNA DSBs (Yan et al. 2007). However, the interaction between RAP80 and BRCA1 is not direct. Rather, it is mediated by a coiled-coil domain-containing protein, Abraxas (also known as CCDC98 and FAM175A), which is phosphorylated at Ser406 and binds to the BRCT domains of BRCA1 (Wang et al. 2007).

Table 1. The mains functions of BRCA1 in DNA damage response and repair, and the associated proteins involved in each step

Function of BRCA1	**Associated proteins**	**References**
Amplification of DNA damage signals	H2AX, MDC1, RNF8, RNF168, UBC13, RAP80	Huen et al. 2009; Stucki et al. 2005; Wang and Elledge 2007; Kolas 2007; Doil et al. 2009; Kim 2007
Activation of cell cycle checkpoint	RAP80, abraxas, BRCC36, BRCC45, MERIT40	Kim 2007; Wang 2007; Liu et al. 2007; Dong 2003; Feng 2009
Double-strand break end resection	CtIP, EXO1, BLM helicase, MRN complex	Sartori 2007; Huertas and Jackson 2009; Mimitou and Symington 2008; Chen 2008
Loading RAD51 recombinase	BRCA2, PALB2, BRCC36, BRCC45	Chen et al. 1998; Dong 2003; Sy et al. 2009; Zhang 2009

Once being recruited to DSB sites, BRCA1 is involved in several processes to facilitate homologous recombination repair (Table 1). The first step is DNA end resection, which initiates HR while suppresses NHEJ (Symington and Gautier 2011). DNA end resection is orchestrated by the coordination of several proteins including the MRN complex, DNA replication helicase/nuclease 2 (DNA2), the exonuclease EXO1 and the Bloom syndrome protein (BLM) helicase (Symington and Gautier 2011; Lavin 2004; Sartori et al. 2007). Whether BRCA1 is required for DNA end resection, however, is unclear at this moment due to apparently contradictory data from different studies (Reczek et al. 2013; Yun and Hiom 2009). The MRN complex initiates a short (50–500 nt) DNA end clipping from the 5' end in conjunction with CtIP (Sartori et al. 2007; Paull and Gellert 1998). Subsequently, a more extensive (up to 5 kb) resection is carried out by DNA2, EXO1 and BLM helicase to generate longer 3' ssDNA overhangs (Feng et al. 2013). CtIP physically interacts with MRE11 and is also recruited to the BRCA1 BRCT domains after phosphorylation at Ser327 (Yu and Chen 2004). This raises the hypothesis that BRCA1 might be involved in DNA end resection as well. Indeed, with a BrdU incorporation assay to assess ssDNA formation, Schlegel et al. (2006) showed that BRCA1 promoted ssDNA foci formation after ionization radiation. Moreover, Chen et al. (2008) observed that accumulation of ssDNA/RPA foci, detected by a RPA-specific antibody, was reduced in

BRCA1-mutant HCC1937 cells after ionization radiation. Re-expression of wildtype BRCA1 restored the generation of ssDNA coated by RPA, suggesting that BRCA1 is required for efficient end resection. However, a study by Zhao et al. (2007) showed that after IR, RPA could form foci in most Hela cells depleted for BRCA1, indicating that BRCA1 is not required for DNA end resection. In addition, studies by Nakamura et al. (2010) and Reczek et al. (2013) showed that CtIP-dependent end resection did not require its interaction with BRCA1. In contrast, another study showed that a mutant CtIP which cannot interact with BRCA1 due to de-phosphorylation of Ser327 caused decreased levels of ssDNA generation and defective homologous recombination (Yun and Hiom 2009). Taken together, these results suggest that the role of BRCA1 in end resection following DNA DSBs needs further clarification.

A more established role of BRCA1 in DNA damage repair is to load RAD51 recombinase to ssDNA after DSB end resection, which is required for strand exchange in homologous recombination (Figure 4). ssDNA generated after DSB end resection is coated by RPA to prevent the formation of hairpins (Chen et al. 2013), which is then displaced by RAD51 with the help of several factors to form a nucleoprotein filament (Sung and Robberson 1995). It was shown that BRCA1 is required for DSB localization of both BRCA2 and RAD51 after microirradiation (Greenberg et al. 2006). Furthermore, BRCA2 is required for the assembly of RAD51 complex following ionization radiation (Davies et al. 2001). Together with previous findings which showed that both BRCA1 and BRCA2 interact with RAD51, these experiments suggest that RAD51 is recruited to DSB sites through a BRCA1-BRCA2 cascade, which highlights the common roles of BRCA1 and BRCA2 in regulating HR repair and maintaining genomic integrity. Further studies demonstrate that BRCA1 and BRCA2 do not interact directly; rather, they are bridged by PALB2, which interacts directly with BRCA1 and is required for loading BRCA2-RAD51 to the DSB sites (Sy et al. 2009; Zhang et al. 2009). In line with its role in linking BRCA1 and BRCA2, thus facilitating DNA damage repair, PALB2 was found to be a breast cancer susceptibility gene (Rahman et al. 2007). After RAD51 binds to ssDNA, the nucleoprotein filament starts to search and invade the homologous sequence, which forms a D-loop structure (Bianco et al. 1998). The D loop can be unwound and the freed ssDNA strand anneals with the strand associated with the other DSB end. This reaction is completed by gap-filling DNA synthesis and re-ligation, which produces only noncrossover sequences (San Filippo et al. 2008). The D loop may also form two Holliday junctions after the second DSB end is captured by the invaded ssDNA, accompanied by gap-filling DNA synthesis and re-ligation (San Filippo et al. 2008). The two Holliday junctions can be resolved to form crossover or noncrossover sequences, or they can be dissolved to form exclusively noncrossover sequences (Matos and West 2014).

1.3.4 BRCA1 regulates cell cycle checkpoints

An actively proliferating cell is going through a series of events that lead to its division, which produces two daughter cells. The whole process is termed a cell cycle. A full cell cycle can be divided into four phases that do not have clear boarders: gap1 (G1 phase), synthesis (S phase), gap2 (G2 phase) and mitosis (M phase) (Cooper 2000). The first three phases are collectively called interphase, during which a cell replicates its DNA and prepares for division, which takes place in M phase and produces two daughter cells that enter interphase again. In order to pass genetic information to daughter cells faithfully and ensure proper cell division, cells have evolved sophisticated control mechanisms for each step of the cell cycle. These control mechanisms, termed cell cycle checkpoints, can be activated by DNA damage to temporarily stop cell proliferation at different stages. This prevents the cells from dividing with damaged DNA, which would cause either genetic mutations or apoptotic cell death (Smith et al. 2010). Depending on the stage of a cell upon DNA damage, it can be arrested at G1 phase (G1 checkpoint), S phase (intra-S checkpoint) or G2/M phase (G2/M checkpoint). Current data suggest that BRCA1 is involved in the control of all three checkpoints, often together with the complexes that regulate DNA damage repair, highlighting BRCA1 as a multifunctional protein in maintaining genome integrity through coordination with other factors (Deng 2006).

G1 checkpoint: G1 cell cycle checkpoint (also known as restriction point) is mainly controlled by the tumor suppressors Rb and p53 (Weinberg 2007). Activated G1 cell cycle checkpoint delays cells to enter S phase and start DNA replication. During G1 phase, activated Rb is hypophosphorylated, which binds to and inhibits the transcription factors of E2F family to prevent G1–S transition (van den Heuvel and Dyson 2008). When cells transit from G1 to S phase, Rb is inactivated by phosphorylation, which releases and activates E2F transcription factors. The E2F family members E2F1–3 transcribe the expression of several genes required for the G1–S transition, including cyclin E, which forms a complex with CDK2 (Nevins 2001). p53 plays a central role in controlling G1 cell cycle checkpoint, partly by increasing the expression of its downstream target p21, which acts as an inhibitor of G1–S promoting complex CDK2/cyclin E (Bartek and Lukas 2001).

The main factors responsible for the regulation of cell cycle checkpoints in response to DNA damage consist of ATM-CHK2 and ATR-CHK1 pathways (Smith et al. 2010). Upon DNA damage induction, activated ATM phosphorylates CHK2 at Thr68, which leads to its transient homodimerization, intermolecular autophosphorylation and full activation (Ahn et al. 2002; Cai et al. 2009). Activated CHK2 then phosphorylates a number of substrates, including p53, CDC25 phosphatases and BRCA1, which is required for G1 checkpoint activation (Chehab et al. 2000; Zhang et al. 2004). Activated ATM also phosphorylates multiple sites of MDM2, leading to its inactivation and in turn stabilization

of p53, which is then phosphorylated by CHK2 at Ser20 (Chehab et al. 2000; Chen et al. 2005). p53 is also directly phosphorylated by ATM and ATR at Ser15, and at S18 by CHK1 following its activation by ATR (Canman et al. 1998; Banin et al. 1998). All of these contribute to the activation of p53 and increased expression of p21. BRCA1 contributes to G1 checkpoint activation partly by regulating ATM/ATR activity. A study by Fabbro et al. (2004) showed that depletion of either BRCA1 or its partner BARD1 by siRNA inhibited ATM/ATR-mediated phosphorylation of p53 at Ser15, thus preventing the activation of G1 cell cycle checkpoint following IR-induced DNA damage. BRCA1/BARD1-dependent phosphorylation of p53 is unique since phosphorylation of other ATM/ATR substrates, including CHK1, CHK2 and H2AX, was not affected after depletion of BRCA1 or BARD1 (Fabbro et al. 2004). Moreover, they found that BRCA1 is a direct substrate of ATM/ATR, and its phosphorylation at Ser1423 and Ser1524 is required for the adaptor function to mediate p53 phosphorylation. This study put BRCA1 in the canonical pathway of G1 cell cycle checkpoint, which is controlled by the ATM–CHK2–p53–p21 axis. In addition, BRCA1 can induce the expression of p21 in a p53-independent manner following DNA damage (Somasundaram et al. 1997). This is achieved directly through the transcriptional activity of BRCA1, which can bind to the promoter of p21. Therefore, expression of BRCA1 inhibits cell cycle progression only in p21-wildtype but not p21-deficient cells.

BRCA1 also controls G1 cell cycle checkpoint by regulating the activity of Rb. A study by Aprelikova et al. (1999) showed that only Rb-wildtype cells were sensitive to BRCA1-mediated G1 cell cycle arrest. At the molecular level, they found that BRCA1 interacts with hypophosphorylated Rb and keeps it in an active state, which arrests cells at G1 phase through inhibition of E2F transcription factors.

Intra-S-phase checkpoint: Compared with the well-studied functions of BRCA1 in G1 checkpoint control, its role in regulating intra-S checkpoint is somewhat ambiguous. This might be partly due to the unique characters of intra-S checkpoint itself, which is usually transient upon DNA damage (Kastan and Bartek 2004; Bartek et al. 2004). If damaged DNA is not repaired during the short period of intra-S arrest, cells will continue to exit S phase and arrest at G2 phase (Bartek et al. 2004). Under normal conditions, proper control of S phase involves regulation of both replication origin firing and replication fork progression (Petermann et al. 2010; Izawa et al. 2011). Collision of DNA polymerases with DNA lesions blocks replication progression and generates replication stress, which may lead to fork collapse if the replisome components are not stabilized. The intra-S cell cycle checkpoint is generally thought to be independent of p53, but controlled by two parallel pathways branching from ATM/ATR activation: ATM–MDC1–MRN–SMC1 and ATM/ATR–CHK1/CHK2–CDC25A (Bartek et al. 2004; Yazdi et al. 2002). Structural maintenance of chromosomes (SMC1) associates with BRCA1 and is phosphorylated in an

ATM- and NBS1-dependent manner, which is required for ATM-mediated intra-S-phase checkpoint control (Yazdi et al. 2002; Kitagawa et al. 2004). Under normal conditions, CDC25A contributes to cell cycle progression by removing the inhibitory phosphate groups from cyclin-dependent kinase CDK2 (Sampath et al. 2002). Following DNA damage, phosphorylation of CDC25A by CHK1/CHK2 leads to its ubiquitination and degradation and, as a result, arrests cells at S phase (Xiao et al. 2003).

Several studies suggest that a functional BRCA1 contributes to the intra-S-phase checkpoint control. A study by Xu et al. (2002) showed that mutation of BRCA1 at Ser1387, a target of ATM phosphorylation, abrogated the IR-induced S-phase arrest, but not G2/M arrest. On the other hand, the IR-induced G2/M arrest is compromised by mutation of another ATM-targeting residue of BRCA1, Ser1423, which does not affect the intra-S checkpoint. BRCA1 is also involved in the regulation of the intra-S checkpoint induced by stalled replication forks, which activate ATR–CHK1 pathway. Tibbetts et al. (2000) demonstrated that phosphorylation of BRCA1 after treatment with UV light or DNA replication inhibitor was ATM independent, but dependent on ATR. Moreover, activated ATR following HU or UV treatment relocalizes to form nuclear foci overlapping with BRCA1. In addition, BRCA1 contributes to stabilizing stalled replication forks caused by DNA damage, which requires activation of intra-S checkpoint (Schlacher et al. 2012; Hu et al. 2012).

G2/M checkpoint: The G2/M checkpoint has a profound role in response to DNA damage. Defective G2/M checkpoint allows cells to enter mitosis with damaged DNA, which triggers an attempt of aberrant chromosome segregation, leading to the activation of apoptotic pathway (Vitale et al. 2011). If cells survive due to dysfunctional surveillance mechanisms, damaged DNA will be accumulated, which leads to genomic instability and cancer development. The G2/M checkpoint is largely controlled by blocking the activation of CDK1/cyclin B complex, which promotes the transition of cells from G2 to M phase under normal conditions (DiPaola 2002). Activation of CDK1 is achieved through removing the inhibitory Thr14/Tyr15 phosphates by CDC25 phosphatases, whose activities are controlled by phosphorylation-dependent ubiquitination followed by degradation (DiPaola 2002; O'Connell et al. 2000). CDK1 is phosphorylated by WEE1 at the sites of Thr14 and Tyr15, leading to its inactivation. As a result, the activity of CDK1 is controlled by the balance between CDC25 phosphatases and WEE1 kinase (DiPaola 2002).

BRCA1 participates in G2/M checkpoint control partly by regulating phosphorylation of CHK1 (Yarden et al. 2002). Previous studies already indirectly linked BRCA1 with CHK1 phosphorylation in response to DNA damage-induced G2/M checkpoint control. Upon DNA damage induced by IR, MDC1 is required for the localization and phosphorylation of BRCA1, as well as phosphorylation of CHK1, which

in turn is required for cell cycle checkpoint activation (Lou et al. 2003; Stewart et al. 2003). In line with these studies, Yarden et al. (2002) showed that BRCA1 is required for activating CHK1 through phosphorylation upon DNA damage, which in turn contributes to the arrest of cells at G2/M phase. They further showed that BRCA1 controls the expression and phosphorylation of CDC25C and CDK1/cyclin B1 through regulating the expression of WEE1 and 14-3-3 family proteins. Upon DNA damage, increased expression of 14-3-3 family proteins sequesters phosphorylated CDC25C and CDK1/cyclin B in cytoplasm, and thus keeps them in an inactive state (Lopez-Girona et al. 1999).

As mentioned above, mutant BRCA1 that cannot be phosphorylated at Ser1423 abrogates G2/M checkpoint in response to DNA damage induced by IR, but does not affect the intra-S checkpoint (Xu et al. 2002). Using a BRCA1-mutant cell line, HCC1937, which exhibits G2/M checkpoint abnormality, the same group showed that re-expression of wildtype BRCA1 restored IR-induced G2/M arrest (Xu et al. 2001). Expression of a mutant BRCA1, in which the ATM phosphorylation site Ser1423 was mutated to Ala, however, could not rescue the defect of G2/M arrest. These results indicate the requirement of different phosphorylation sites of BRCA1 in regulating different cell cycle stages.

Phosphorylation of BRCA1 by CHK2 is also involved in the regulation of G2/M checkpoint. Prior to DNA damage, CHK2 interacts and colocalizes with BRCA1 within discrete nuclear foci (Lee et al. 2000). IR-induced DNA damage separates BRCA1 from CHK2, which requires phosphorylation of BRCA1 at Ser988 by CHK2. In order to study the functions of BRCA1 phosphorylation at Ser988, Kim et al. (2004) mutated the equivalent residue (Ser971) in mouse Brca1. They found that the Brca1-mutant cells displayed reduced ability to activate G2/M checkpoint in response to γ-radiation, as well as reduced p53 stabilization after MNNG treatment. In another study with mouse embryonic fibroblasts (MEFs) derived from targeted deletion of exon 11 of the *Brca1* gene, Xu et al. (1999) demonstrated that the mutant cells had a defective G2/M checkpoint accompanied by extensive chromosome abnormalities. Taken together, these results indicate that BRCA1 plays important roles in regulating G2/M cell cycle checkpoint, which contributes to its functions in maintaining genomic integrity.

Accumulating evidence suggests that centrosome integrates cell cycle checkpoint and repair signals in response to DNA damage (Löffler et al. 2006). One important mediator of this response, CHK1, has been shown to negatively regulate the G2–M transition via its centrosomal localization (Krämer et al. 2004). The BRCA1/BARD1 complex was found to localize at the centrosome throughout the cell cycle, where it ubiquitinates γ-tubulin at the residues of Lys-48 and Lys-344, which is required for centrosome duplication (Sankaran et al. 2005; Sankaran et al. 2006). This raises the possibility that the function of BRCA1 in controlling centrosome duplication might contribute to its role in the regulation of G2/M

cell cycle checkpoint. An earlier study by Xu et al. (1999) isolated mouse embryonic fibroblasts carrying a targeted deletion of exon 11 of the *Brca1* gene. In line with the hypothesis, they found that these MEFs had a defective G2/M checkpoint accompanied by extensive chromosomal abnormalities caused by multiple centrosomes.

1.3.5 The role of BRCA1 in development and stem cell regulation

Similarities in higher risks of breast cancer development upon of BRCA1 and BRCA2 mutations can be explained by their shared functions in keeping genomic stability. However, BRCA1-mutant breast tumors have distinct phenotypes compared with those caused by BRCA2 mutations. Moreover, a large number of sporadic breast cancers are observed with methylation of *BRCA1* promoter, which inhibits the expression of BRCA1 protein (Hsu et al. 2013). Interestingly, these breast cancers tend to have a basal-like phenotype as well, with low or no expression of estrogen receptor (Bal et al. 2012; Hsu et al. 2013). Consistent observations between BRCA1 mutations and basal-like phenotypes suggest that BRCA1 may play a role in mammary gland development and, hence, its loss of function controls the phenotypes of breast tumors.

There is strong evidence suggesting that luminal and basal cells are differentiated from a common cell of origin: the mammary stem cells, which are basal-like and ER negative (Smalley and Ashworth 2003; Turner et al. 2004). To explain the association of BRCA1 mutations and the basal-like phenotype, Foulkes (2004) hypothesized that BRCA1 might be a stem cell regulator which controls the orderly transition of ER-negative stem/progenitor cells to ER-positive epithelial cells. According to this hypothesis, loss of BRCA1 leads to the persistence of basal-like stem cells, which accumulate DNA damage and gradually become cancerous after surviving programmed cell death (Foulkes 2004; Smalley et al. 2008). To counter increased DNA damage due to loss of BRCA1 function, cells need to shut off proapoptotic signals, e.g. by mutating p53. This may explain why BRCA1-mutant breast tumors almost always have mutated p53 (Turner and Reis-Filho 2013). This hypothesis was partly supported by the isolation of breast cancer stem cells, which express basal-like markers, e.g. cytokeratin 5/6 (Al-Hajj et al. 2003; Dontu et al. 2003). In stem cells, proliferation is tightly controlled, while loss of this control is a key feature of cancer. BRCA1-mutant breast cancers usually happen at relatively early ages compared with those caused by BRCA2 mutations, suggesting that cells harboring BRCA1 mutations are readily losing their tight control of proliferation and becoming cancerous (Foulkes 2004). In addition, breast cancers associated with BRCA1 mutations grow quickly and have poor prognosis. All of these phenomena fit the cancer stem cell model, which proposes that cancer arises directly from deregulated stem cells (Dalerba et al. 2007; Allan et al. 2007).

Recently, several studies have shown that BRCA1-mutant breast cancer originates from luminal progenitor cells rather than from basal stem cells, which challenges the cancer stem cell model (Lim et al. 2009; Molyneux et al. 2010). Using CD49f (α6 integrin) and epithelial cell adhesion molecule EpCAM (CD326) as markers, Lim et al. (2009) isolated three epithelial subpopulations from normal mammary tissue and *BRCA1*-heterozygous pre-neoplastic specimens. Surprisingly, they found that luminal subpopulations were enriched in the BRCA1-mutant breast tissues. Moreover, analysis of gene expression profiles revealed that BRCA1-heterozygous tissues, as well as basal-like breast tumors, were more similar to the normal luminal progenitor cells, suggesting that the basal-like breast cancers originate from the luminal progenitor cells (Lim et al. 2009). Indeed, deletion of Brca1 in mouse mammary luminal progenitor cells resulted in basal-like breast tumors. In contrast, depletion of Brca1 in basal stem cells produced tumors that are histologically different to basal-like tumors (Molyneux et al. 2010). In the same study, the receptor tyrosine kinase c-kit emerged as a key marker of luminal progenitor cells and is highly expressed in BRCA1-mutant breast tumors. These data suggest a function of BRCA1 in controlling the differentiation of normal breast tissue. Thus, loss of BRCA1, due to either genetic mutation or epigenetic silencing through its promoter methylation, leads to a strong association with basal-like breast cancer phenotype.

1.3.6 Therapeutic implications of BRCA1 mutations for cancer treatment

Considering the high penetrance of BRCA1 mutations in breast and ovarian cancers, much effort has been made to develop therapeutic strategies for the treatment of cancers associated with BRCA1 mutations. Since the main function of BRCA1 is to facilitate HR repair together with BRCA2 and several other proteins, the therapeutic strategies are usually applicable to the treatment of cancers caused by mutations of other genes involved in HR pathway.

A breakthrough came when two groups independently found that BRCA1/2-deficient cells are more sensitive to inhibitors targeting poly (ADP-ribose) polymerase (PARP) (Farmer et al. 2005; Bryant et al. 2005). PARP is a family of enzymes involved in BER/SSBR, a key pathway required for repairing DNA single-strand breaks (Rouleau et al. 2010). When PARP is inhibited, unrepaired SSBs are converted to DSBs when they encounter replication forks (Schreiber et al. 2006; Rouleau et al. 2010). In normal cells, PARP inhibition-induced DSBs can be repaired by homologous recombination pathway with the help of functional BRCA1 and BRCA2. In cancer cells deficient for BRCA1 or BRCA2, however, dysfunctional HR leads to accumulation of massive DSBs, which induce apoptotic cell death (Farmer et al. 2005; Bryant et al. 2005). Based on this theory, cells with mutations of other HR components should be more sensitive to PARP inhibitors as well. Indeed, several studies have demonstrated that PARP inhibitors can selectively kill

cancer cells deficient for other HR components, e.g. PALB2 and ATM (Buisson et al. 2010; McCabe et al. 2006; Turner et al. 2008). Several PARP inhibitors are under development to target BRCA1/2-mutant breast and ovarian cancers, some of which have gained promising results in clinical trials (Audeh et al. 2009; Fong et al. 2009). Olaparib (AZD2281) is an orally active PARP inhibitor that is highly potent against both PARP1 and PARP2 (Menear et al. 2008). A phase II clinical trial compared the efficacy and safety of olaparib and pegylated liposomal doxorubicin (PLD) in patients with recurrent ovarian cancer and BRCA1 or BRCA2 mutations (Kaye et al. 2012). The result showed consistent efficacy of olaparib compared with previous studies, despite that there was no significant difference between olaparib and PLD in the primary end point of progression-free survival (PFS). Then it was studied in a phase III clinical trial to test its benefit as a maintenance monotherapy in patients with BRCA1/2-mutant ovarian cancer (ClinicalTrials.gov Identifier: NCT01844986). After a successful clinical trial, olaparib was approved in December 2014 by FDA for the treatment of ovarian cancers associated with BRCA1/2 mutations. Other PARP inhibitors that are under development *in vitro* or in clinical trials include NU1025, AG14361, rucaparib (AG014699) and veliparib (ABT-888) (Isakoff et al. 2010; Drew et al. 2011).

Preclinical studies have found that BRCA1- or BRCA2-deficient cells are more sensitive to other DNA-damaging agents, notably platinum drugs and mitomycin C (Turner et al. 2004). Both cisplatin and mitomycin C induce RAD51 foci localized in nuclei, a marker of homologous recombination repair (Al-Minawi et al. 2009). As a result, cells derived from BRCA1/2-mutant tumors die due to persistent DNA damage induced by cisplatin or mitomycin C (Bhattacharyya et al. 2000; Alli et al. 2011). In fact, several clinical studies have already demonstrated that women with BRCA1/2-mutant ovarian cancers have better prognosis than those with wildtype BRCA1/2 when treated with platinum drugs (Boyd et al. 2000; Foulkes 2006).

Despite solid preclinical studies and promising clinical trials, resistance of BRCA1/2-mutant tumors to the abovementioned drugs occurs in many cases (Lord and Ashworth 2013). Some BRCA1/2-mutant tumors have shown resistance from the beginning, while others respond to PARP inhibitors or cisplatin initially, but gain resistance after certain time. Several mechanisms for increased resistance to PARP inhibitors and cisplatin have been identified or proposed. Interestingly, in BRCA1/2-mutant ovarian cancers treated with a PARP inhibitor, there is an association between the clinical benefit rate and platinum-free interval (Fong et al. 2013). This suggests that there might be common mechanisms rendering BRCA1/2-mutant tumors resistant to both PARP inhibitors and platinum. For example, several groups have shown that functions of mutated BRCA1/2 can be partially restored by secondary mutations that shift the open reading frames, which

rescue the DNA repair function and confer resistance to both PARP inhibitors and cisplatin (Sakai et al. 2008; Swisher et al. 2008). Other resistance mechanisms include increased expression of P-glycoprotein efflux pumps or decreased expression of 53BP1 (Rottenberg et al. 2008; Bouwman et al. 2010). The resistance of cancer cells to certain therapeutics reveals their high heterogeneity; and more research needs to be done to uncover the molecular mechanisms in order to increase therapeutic efficacy.

2. Aims of the study

The first aim of the study was to better understand the biological nature of BRCA1-mutant breast cancer. We argued that phenotypes of BRCA1-mutant breast cancer cell lines could reflect the characteristics of corresponding tumors in clinics. Therefore, we collected four BRCA1-mutant breast cancer cell lines for their phenotypical characterization.

The second aim of the study was to identify potential targets that are synthetic lethal with BRCA1 deficiency. For the purpose of this, we carried out a high-throughput screen with a library of cancer-related drugs on a pair of BRCA1- and control-knockdown cells.

A long-standing question regarding BRCA1 functions is why BRCA1 mutations almost always cause basal-like subtype of breast cancer. In order to answer this question, we established an *in vitro* model of mouse mammary epithelial cells (mMECs), which was used to study the differentiation of mMECs from luminal to basal lineages.

3. Materials and methods

3.1 Antibodies and drugs

Antibodies and drugs used in the experiments are listed in Table 2 and Table 3, respectively.

Table 2. List of antibodies and their related information used in this study

Antibody	Source	Catalog no.	Vendor	Applications
BRCA1 (C-20)	Rabbit	sc-642	Santa cruz	IF 1:500
BRCA1 (Ab-1) (MS110)	Mouse	OP92	EMD Millipore	WB 1:300
BRCA2 (Ab-1) (2B)	Mouse	OP95	EMD Millipore	WB 1:1000
53BP1	Rabbit	ab21083	Abcam	WB 1:2000; IF 1:1000
gamma H2AX (phospho S139)	Mouse	ab22551	Abcam	WB 1:5000; IF 1:2000
RAD51 (H-92)	Rabbit	sc-8349	Santa cruz	IF 1:500
Cleaved PARP (Asp214)	Rabbit	#9541	Cell Signaling Technology	WB 1:2000
Rb (4H1)	Mouse	#9309	Cell Signaling Technology	WB 1:2000
Phospho-Rb (Ser807/811)	Rabbit	#9308	Cell Signaling Technology	WB 1:1000
Phospho-Histone H3 (Ser10) (D2C8)	Rabbit	#3377	Cell Signaling Technology	IF 1:1600
E2F1 (C-20)	Rabbit	sc-193	Santa cruz	WB 1:1000
cyclin B1 (H433)	Rabbit	sc-752	Santa cruz	WB 1:2000
p53 (DO-1)	Mouse	sc-126	Santa cruz	WB 1:2000
p21 (6B6)	Mouse	554228	BD Biosciences	WB 1:2000
GAPDH (NB615)	Mouse	NB300-285	Novus Biologicals	WB 1:5000
Beta Actin	Rabbit	NB600-505	Novus Biologicals	WB 1:5000
Tubulin (DM1A + DM1B)	Mouse	ab44928	Abcam	WB 1:10000
IRDye 800CW anti-Mouse IgG (H + L)	Goat	926-32210	LI-COR Biosciences	WB 1:10000
IRDye 680 anti-Rabbit IgG (H + L)	Goat	926-32221	LI-COR Biosciences	WB 1:20000

Table 3. List of drugs and their related information used in this study

Drug	Vendor	Catalog no.	Solvent
Bortezomib (PS-341)	Selleck Chemicals	S1013	Water
Carfilzomib (PR-171)	ChemieTek	CT-CARF	Water
Olaparib (AZD2281)	Selleck Chemicals	S1060	DMSO
Veliparib (ABT-888)	Selleck Chemicals	S1004	DMSO
Cisplatin (cis-Diammineplatinum(II) dichloride)	Sigma-Aldrich	P4394	*N*,*N*-Dimethylmethanamide
Mitomycin C	Roche	10107409001	DMSO
Selumetinib (AZD6244)	JS Research Chemicals Trading	MEK-SELU	DMSO
Pimasertib (AS-703026)	Selleck Chemicals	S1475	DMSO
Trichostatin A (TSA)	Selleck Chemicals	S1045	Ethanol
XI-011 (NSC 146109)	R&D Systems	2815	DMSO
Serdemetan (JNJ-26854165)	Selleck Chemicals	S1172	DMSO

3.2 Cell culture

Cell lines HCC1937, MDA-MB-231, MDA-MB-436, MCF-7, Hela, U2OS and MCF-10A were purchased from the American Type Culture Collection (ATCC). HCC1937 was maintained in RPMI 1640, the other five cell lines in DMEM. Both media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1 × Penicillin-Streptomycin (Life Technologies). MCF-10A cells were grown in DMEM/F-12 medium supplemented with 5% horse serum, 20 ng/ml EGF, 0.5 μg/ml hydrocortisone, 0.1 μg/ml

cholera toxin, 10 μg/ml insulin and 1 × Penicillin-Streptomycin. The SUM cell lines were obtained from Dr. John Martens at Erasmus University Medical Center in the Netherlands. They were grown in Ham's F-12 medium containing 5% FBS, supplemented with EGF (10 ng/ml) and insulin (10 μg/ml) for SUM1315MO2, or with hydrocortisone (0.5 μg/ml) and insulin (10 μg/ml) for SUM149PT and SUM229PE. All cell lines were authenticated at FIMM Technology Centre, and maintained at 37 ℃ with 5% CO_2. Generation of *R26*$^{CreERT2/RMCE}$;*Brca1*$^{SCo/\Delta}$ mouse embryonic stem cells expressing human *BRCA1* cDNA or RMCE vector was conducted by our collaborators in the Netherlands Cancer Institute (NKI) as described by Bouwman et al. (2013).

3.3 Gene silencing by siRNAs

siRNAs were purchased from Qiagen. They were transfected with Lipofectamine RNAiMAX Transfection Reagent (Life Technologies) according to the manufacturer's protocol. For high-throughput chemical compound screening, MDA-MB-231 cells were transfected with BRCA1 or control siRNA at the final concentration of 20 nM 24 h before the screen. For all other applications, cells were transfected with siRNAs at the final concentration of 10 nM one day before the experimental assays.

3.4 Drug sensitivity tests

Cells were trypsinized and resuspended in corresponding media, seeded in 96-well plates. Each drug was diluted into a series of concentrations in culture medium and a 50 μl solution was added to each well of the plates. After treating cells with each drug for 3 days, CellTiter-Blue (Promega) was added to reach a final concentration of 10%, incubated for 3 hours and the fluorescent signals were measured by PHERAStar Plus (BMG Labtech) plate reader. Cell viability curves were plotted in Microsoft Excel, and the half maximal inhibitory concentration (IC50) of each drug for each cell line was calculated from the trend line fit to each curve. The drug sensitivity curves for MEK inhibitors selumetinib and pimasertib (AS-703026), and proteasome inhibitors bortezomib and carfilzomib were plotted with GraphPad Prism. For bortezomib sensitivity assay in *R26*$^{CreERT2/RMCE}$;*Brca1*$^{SCo/\Delta}$ mouse ES cells, endogenous *Brca1*^{SCo} allele was deleted by incubating the cells with 0.5 μM 4-hydroxytamoxifen (4-OH) overnight. After one week, cells were seeded in 96-well plates at 1000 cells/well for the drug sensitivity test.

3.5 High-throughput screening

Drugs from the library of FIMM Oncology (Appendix I, supplementary Table S2) were dissolved in corresponding media and dispensed into five 384-well plates, 5 μl/well. Cells were trypsinized, resuspended and added in the five plates with a multidrop dispenser (Thermo Scientific), 20 μl/well. After treating cells for 3 days, cell viabilities were measured with CellTiter-Blue Cell Viability Assay (Promega) as described above. Dose–

response curves and the medium lethal doses (LD50s) were generated with Studies software (Dotmatics Limited) as described by Pemovska et al. (2013).

3.6 Western blot

After removing the media, cells cultured in 6-cm dishes were washed once with 1 × PBS, lysed with modified RIPA buffer (50 mM Tris-HCl pH8.0, 250 mM NaCl, 2 mM EDTA, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 5 mM NaF and 5 mM Na_3VO_4) supplemented with a protease inhibitor tablet (Thermo Scientific). Cell lysates were agitated for 30 min at 4 ℃, followed by high-speed centrifugation for 10 min. The supernatants were collected and quantified with BCA Protein Assay Kit (Pierce). 50 μg of each cell lysate was mixed with NuPage SDS Loading Buffer for electrophoresis, which was carried out with NuPAGE Novex 4–12% Bis-Tris Protein Gels (Life Technologies), followed by blotting to nitrocellulose membranes (Millipore). The membranes were incubated overnight with primary antibodies diluted in blocking buffer (5% milk in 1 × TBST), followed by IRDye secondary antibodies (LI-COR Biosciences) for 1 hour at room temperature. After 3 times washing with 1 × TBST, membranes were scanned with Odyssey infrared imaging system (LI-COR Biosciences).

3.7 Immunofluorescence

Cells were seeded on coverslips in 24-well plates 36 hours before experimental assays. For measuring the HR capability of each cell line, cells were treated with IR at 4 Gy and incubated for 6 hours before staining. For detecting DNA damage, Hela cells were treated with bortezomib or 5 Gy IR and incubated for 8 hours before staining. After washing with 1 × PBS, cells were fixed with 2% paraformaldehyde diluted in PBS^{++} (1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ in 1 × PBS) for 15 min. The cells were then washed 3 times with PBS^{++}, followed by permeabilization with 0.5% Triton X-100 in PBS^{++} for 15 min. After that, cells were washed 3 times with incubation buffer (0.5% BSA, 0.15% glycine, 0.1% Triton X-100 in 1 × PBS) and blocked for 30 min. Cells were co-stained with mouse-anti-γH2AX and rabbit-anti-RAD51 for HR capacity measurement. For detecting DNA damage, cells were co-stained with mouse-anti-γH2AX plus rabbit-anti-BRCA1 or rabbit-anti-53BP1. Cells were incubated with primary antibodies overnight at 4 ℃, followed by 3 times washing with incubation buffer. Then they were co-stained with secondary antibodies labeled with Alexa488 or Alexa594 for 1 hour at room temperature. Cell nuclei were stained with Hoechst 33258. Images were taken with Nikon Eclipse 90i fluorescent microscope.

3.8 Flow cytometry

Cell cycle distribution was analyzed by staining cell nuclei with the Propidium Iodide Staining Solution (eBioscience) according to the protocol provided with the reagent.

Briefly, cells seeded in 6-well plates were resuspended and washed with cold PBS, followed by fixation with cold 70% ethanol overnight. Propidium iodide (PI) staining was carried out by resuspending cells in 500 μl PI/Triton X-100 solution (to 10 ml of 0.1% Triton X-100 in PBS add 2 mg DNase-free RNase A and 50 μl PI Staining Solution) for 30 min at room temperature. Apoptotic cells were detected by using Annexin V Apoptosis Detection Kit (eBioscience) according to the manufacturer's protocol. Briefly, cells were resuspended in 1 × Binding Buffer after treatment as indicated in each experiment. Then 5 μl of APC-conjugated Annexin V was added to 100 μl of the cell suspension, incubated for 15 min at room temperature. After that, cells were washed twice and resuspended in 500 μl 1 × Binding Buffer for flow cytometry analysis. Flow cytometry was performed in Accuri C6 flow cytometer and data analyzed with CFlow Sampler (BD Biosciences).

3.9 Proteome profiling assay

Proteome profiling assays were performed according to the protocols provided with the Proteome Profiler Antibody Arrays (R&D Systems). Briefly, cells were lysed directly in 10-cm dishes after washing once with 1 × PBS. The cell lysates were then agitated, centrifuged to remove cell debris and quantified with BCA Assay Kit. 300 μg of each cell lysate was added to the pre-blocked membranes and rocked gently overnight at 4 ℃. The membranes were then washed 3 times and incubated with reconstituted Detection Antibody Cocktails for 2 hours at room temperature. After washing 3 times, the membranes were incubated with diluted Streptavidin-HRP for 30 min and detected with enhanced chemiluminescence (ECL, Bio-Rad).

4. Results and discussion

4.1 Heterogeneity of BRCA1-mutant breast cancer cell lines (Appendix III)

As breast cancer cell lines are established from spontaneously developed breast tumors, their phenotypic characteristics can reflect the biological nature of corresponding tumors. Therefore, we collected and characterized four breast cancer cell lines bearing different BRCA1 mutations, namely HCC1937, MDA-MB-436, SUM1315MO2 and SUM149PT. Mutations of *BRCA1* and several other key genes thought to play pathogenic roles in cancer development, including *TP53*, *PTEN*, *RAS*, *PI3K* and *BRAF*, are listed in Table 4. We first tested their sensitivities to several DNA-damaging agents, followed by measuring their homologous recombination capacities. Then we carried out a high-throughput chemical compound screen, as well as a proteome profiling assay to measure the kinase activities of these cells. Because BRCA1-mutant breast cancer tends to have a basal-like phenotype, we used two basal-like breast cancer cell lines that express wildtype BRCA1 in drug sensitivity tests, MDA-MB-231 and SUM229PE. In other experiments, we also included MCF-7, a luminal breast cancer cell line expressing wildtype BRCA1 and p53; as well as MCF-10A, which is an immortalized mammary epithelial cell line with a stable, near-diploid karyotype and few genetic modifications (Soule et al. 1990; Yaswen and Stampfer 2002).

Table 4. Mutations of the cell lines used in this study

Cell lines	*BRCA1* mutation	*TP53* mutation	*PTEN* mutation	*KRAS* mutation	*RAF* mutation	*PIK3CA* mutation
HCC1937	5382insC	mutant	deletion	wildtype	wildtype	wildtype
MDA-MB-436	5396 + 1G>A	mutant	wildtype	wildtype	wildtype	wildtype
SUM1315MO2	185delAG	mutant	wildtype	wildtype	wildtype	wildtype
SUM149PT	2288delT	mutant	wildtype	wildtype	wildtype	wildtype
SUM229PE	wildtype	mutant	wildtype	mutant	wildtype	wildtype
MDA-MB-231	wildtype	mutant	wildtype	mutant	mutant	wildtype
MCF-7	wildtype	wildtype	wildtype	wildtype	wildtype	wildtype
MCF-10A	wildtype	wildtype	wildtype	wildtype	wildtype	wildtype

4.1.1 BRCA1-mutant cells are resistant to DNA-damaging agents

Based on the roles of BRCA1 in DNA damage response and repair, its mutation predicts increased sensitivity to certain DNA-damaging agents, especially those that provoke HR pathway for DNA repair (Gudmundsdottir and Ashworth 2006). The platinum-based anticancer drugs (cisplatin, carboplatin and oxaliplatin) and the aziridine-containing compound mitomycin C are widely used in the treatment of different cancers, including breast cancer (Deans and West 2011). Both cisplatin and mitomycin C mainly induce interstrand crosslinks (ICLs) that may scramble several pathways for repair, including NER, HR, translesion synthesis and Fanconi anemia pathway (Muniandy et al. 2010; Deans and West 2011). Responses of the four BRCA1-mutant and two BRCA1-wildtype cell lines to cisplatin and mitomycin C had similar patterns, indicating similar mechanisms of action of

these two drugs (Figure 6A & B). In addition, the BRCA1-mutant cell lines were generally resistant to both cisplatin and mitomycin C, as indicated by the IC50s of HCC1937 and SUM149PT cell lines, which are almost as high as that of the BRCA1-wildtype SUM229PE cells (Figure 6A & B).

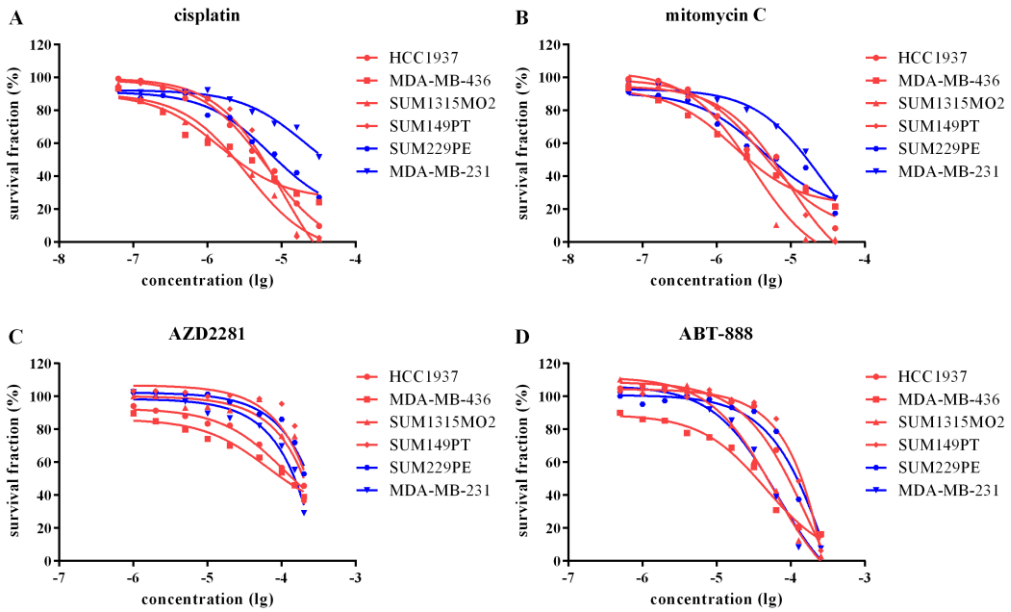

Figure 6. BRCA1-mutant cell lines are resistant to cisplatin, mitomycin C and PARP inhibitors. The BRCA-mutant and BRCA1-wildtype cells were treated with the DNA-damaging agents at a series of concentrations for 3 days, and cell viabilities were measured by the CellTiter-Blue Cell Viability Assay (Promega).

Based on the concept of synthetic lethality, BRCA1/2-deficient cells are more sensitive to inhibitors targeting PARP (Farmer et al. 2005; Bryant et al. 2005). We treated the six cell lines with two PARP inhibitors: olaparib (AZD2281) and veliparib (ABT-888), but their sensitivities seemed not to directly correlate with BRCA1 status. The BRCA1-mutant SUM149PT cells were very resistant to PARP inhibitors, while the BRCA1-wildtype MDA-MB-231 cells were relatively sensitive to both AZD2281 and ABT-888 (Figure 6C & D). The sensitive phenotype of MDA-MB-231 cells observed in our experiment is consistent with published data, in which the authors showed that MCF-7, another BRCA1-wildtype cell line, is also very sensitive to PARP inhibitors (Inbar-Rozensal et al. 2009). They proposed that the hypersensitivity could be attributed to defects of other HR repair factors (Inbar-Rozensal et al. 2009). On the other hand, resistance of BRCA1-mutant tumors to cisplatin and PARP inhibitors has been observed in preclinical studies (Frizzell and Kraus 2009). One of the mechanisms is the restoration of HR due to secondary mutations that correct the open reading frame of *BRCA1* (Norquist et al. 2011).

Other possible mechanisms include the increased expression of P-glycoprotein efflux pumps or decreased expression of 53BP1 (Bouwman et al. 2010; Choi and Yu 2014)

4.1.2 Characterization of homologous recombination efficiency in BRCA1-mutant cells (III)

The best studied function of BRCA1 is to facilitate homologous recombination by loading the recombinase RAD51, thus helping to eliminate damaged DNA. In order to confirm if the BRCA1-mutants are indeed defective for homologous recombination, we performed RAD51 foci formation assay, a standard protocol for measuring HR capacity (Alli et al. 2011). 6 hours after IR treatment at 5 Gy, all BRCA1-wildtype cells formed RAD51 foci, which were colocalized with γH2AX (III, Figure 4). Those cells also formed BRCA1 foci overlapping 53BP1, one of the earliest factors being recruited to DSB sites. The BRCA1 and RAD51 foci observed in untreated cells represent their functions in replication during S phase (Bhattacharyya et al. 2000; Pageau and Lawrence 2006). In contrast to the BRCA1-wildtype cells, three BRCA1-mutant cell lines, HCC1937, MDA-MB-231 and SUM1315MO2, did not form any BRCA1 or RAD51 foci after IR treatment (III, Figure 4). The fourth cell line, SUM149PT, however, formed significantly more RAD51 foci after IR treatment, compared with the untreated cells. In addition, IR-treated SUM149PT cells also formed BRCA1 foci, which were colocalized with 53BP1. SUM149PT cells carry a *BRCA1* mutation of 2288delT, which predicts a shift in *BRCA1* open reading frame (Elstrodt et al. 2006). As a result, it is likely that SUM149PT cells express a partially functional BRCA1 protein, which is enough to form DNA damage foci, and recruit HR repair proteins including RAD51 in response to DNA damage.

Recently, several studies have shown that 53BP1 is required to inhibit homologous recombination in BRCA1-depleted cells by repressing DNA end resection (Bunting et al. 2010; Bouwman et al. 2010). Loss of 53BP1 rescues the phenotypes caused by BRCA1 deficiency (Cao et al. 2009; Bouwman et al. 2010). In order to confirm if 53BP1 is absent in any of the BRCA1-mutant cell lines and thus plays a role in their resistance to the DNA-damaging agents, we detected the expression of BRCA1 and 53BP1 by Western blot. BRCA1 was easily detectable in all of the BRCA1-wildtype cell lines at a molecular weight of 220 kDa (III, Figure 3b). A weak band at the same size was also detected in the BRCA1-mutant HCC1937 cells, consistent with published data (Tassone et al. 2003). This may explain its resistance to the DNA-damaging agents as mentioned above. Expression of BRCA1 was not detectable in other three BRCA1-mutant cell lines, despite the fact that SUM149 cells formed BRCA1 and RAD51 foci after IR treatment. Together with the drug sensitivity tests, these results indicate that different BRCA1-mutant cells may have evolved through different routes during the development of breast cancer, which contributes to the complicated nature of those breast tumors.

4.1.3 High-throughput chemical compound screen in BRCA1-mutant cell lines (III)

Resistance of BRCA1-mutant cells to cisplatin and PARP inhibitors may limit the application of these drugs for the treatment of BRCA1-mutant breast cancer in clinics. Therefore, it is necessary to identify novel therapeutic drugs that can selectively kill BRCA1-deficient cells. For the purpose of this, we carried out a high-throughput screen on BRCA1-mutant and -wildtype cell lines. A locally available library containing 198 cancer-related drugs, termed FIMM Oncology, was used in the screen. Surprisingly, compared with the four BRCA1-wildtype cell lines, all of the four BRCA1-mutant cell lines were found to be more resistant to inhibitors targeting MEK1/2: selumetinib (AZD6244), trametinib (GSK1120212), refametinib (RDEA119) and pimasertib (AS-703026) (III, Figures 1b & 2a). MEK is a dual-specific protein kinase downstream of RAS-RAF signaling pathway. MEK1 and MEK2 mediate phosphorylation of tyrosine and threonine residues in the mitogen-activated protein (MAP) kinases ERK1/2, which contributes to the tumorigenesis of various cancers (Roskoski 2012). Activation of RAF-MEK-ERK pathway predicts increased sensitivity to MEK inhibitors (Solit et al. 2006). Resistance of the BRCA1-mutant cells to MEK1/2 inhibitors, however, indicates that the RAF-MEK-ERK pathway is not hyper-active in these cells. This result also implies that the strategy of targeting MAPK pathway only for the treatment of BRCA1-mutant cancers should be excluded.

The results obtained from drug sensitivity tests, RAD51 foci formation assay and high-throughput chemical compound screen altogether demonstrate diverse phenotypes of the BRCA1-mutant cell lines, which may well represent the heterogeneity of BRCA1-mutant tumors in clinics. In order to get a comprehensive view of the intracellular activities among these cells, we performed a proteome profiling assay with phospho-kinase and phospho-receptor tyrosine kinase arrays. The results showed very diverse kinase activities of different cell lines (III, Figure 2b). Since those cell lines were established from different tumors, their diversity may well reflect the heterogeneity of breast tumors caused by different pathogenetic factors and different environments (Campbell and Polyak 2007).

Careful examination of the kinase activities brought about several common features among a particular group of cell lines. Three of the four BRCA1-mutant cell lines, HCC1937, MDA-MB-436 and SUM149PT, have higher activities of PI3K/Akt pathway, as indicated by Akt phosphorylation at Ser437. The fourth BRCA1-mutant cell line, SUM1315MO2, has no Akt phosphorylation at all, in contrast to other cell lines, which have at least background levels of phospho-Akt (III, Figure 2b & c). Taken together, our data bring about an interesting hypothesis: cells with BRCA1 mutations may rely more on the PI3K/Akt pathway for survival. Inhibition of the RAF/MAPK pathway does not have a significant effect on the survival of these cells, compared with the BRCA1-wildtype cells,

whose survival depends at least partially on the RAF/MAPK pathway. In addition, we also observed higher activities of CHK2 in the basal-like breast cancer cell lines, in comparison with the luminal cell line MCF-7 and the normal cell line MCF-10A. CHK2 is a checkpoint kinase that is usually activated upon DNA damage to arrest cell cycle progression (Smith et al. 2010). This suggests that the basal-like breast cancer cell lines constantly bear high amounts of DNA damage, and thus are highly genomic instable.

4.2 Loss of BRCA1 sensitizes cells to proteasome inhibitors in a DNA repair-independent manner (Appendix I)

4.2.1 Proteasome inhibitors selectively kill BRCA1- but not BRCA2-depleted cells

The extensive heterogeneity of different BRCA1-mutant cell lines is largely attributed to their different genetic backgrounds. This may account for the outcome of the high-throughput screen, which did not yield any synthetic-lethal targets with BRCA1 deficiency. In order to investigate the consequences related to loss of BRCA1 function alone, we carried out a high-throughput screen on a pair of BRCA1- and control-knockdown cells. A basal-like breast cancer cell line, MDA-MB-231, was transfected with either BRCA1 or control siRNA and treated with drugs of the Oncology Library as mentioned above. Two proteasome inhibitors, bortezomib and carfilzomib, were found to be more potent against the BRCA1-knokdown cells than control cells, with the median lethal doses (LD50s) in BRCA1-depleted cells 3 times lower than that of control cells. The selective effects of the two proteasome inhibitors were validated in other two cancer cell lines, Hela and U2OS, in which even more striking sensitization was observed (I, Figure 1a). Surprisingly, depletion of BRCA2, another breast cancer susceptibility gene involved in HR repair, did not sensitize either Hela or U2OS cells to the proteasome inhibitors, suggesting that the sensitizing effect might involve a specific function of BRCA1 that is not related to HR repair (I, Figure 1a).

Next we asked if the sensitizing effect on BRCA1-depleted cells was due to apoptotic cell death induced by proteasome inhibitors. Results from Western blot showed that bortezomib induced expression of cleaved PARP (cPARP) in BRCA1-depleted cells in a dose-dependent manner. Cells depleted for BRCA2 did not show any increase of cPARP, consistent with the BRCA1-specific sensitizing effect (I, Figure 1g). Bortezomib treatment also led to the accumulation of polyubiquitinated proteins in all cells, indicating proteasome inhibition (I, Figure 5g). More importantly, the proteasome inhibitor bortezomib induced stronger effects in the BRCA1-knockdown cells than in BRCA2- or control-knockdown cells, suggesting a special requirement of BRCA1 in antagonizing proteasome inhibition.

To confirm that the selective effect of proteasome inhibitors was due to loss of BRCA1 functions, we utilized mouse embryonic stem cells with inducible *Brca1* knockout,

and re-expressed human *BRCA1* cDNA with the RMCE vector (Bouwman et al. 2013). *Brca1*-knockout mouse ES cells were more sensitive to bortezomib compared with the *Brca1*-heterozygous cells. More importantly, re-expression of human *BRCA1* cDNA in *Brca1*-knockout cells restored their sensitivity to bortezomib almost to the level of *Brca1*-heterozygous cells (I, Figure 1e & f). These results clearly demonstrate that loss of BRCA1 indeed sensitizes cells to proteasome inhibition.

4.2.2 Sensitization of BRCA1-depleted cells to proteasome inhibitors does not involve DNA damage response

Next we sought to investigate the mechanisms through which proteasome inhibitors selectively kill BRCA1-depleted cells. First we asked if this effect was caused by dysfunctional DNA damage repair after loss of BRCA1. To answer this question, we treated Hela cells with bortezomib at a series of concentrations, and measured the amount of γH2AX foci by immunofluorescence after 8 hours. Surprisingly, we did not observe any increased amount of foci for γH2AX, BRCA1 or 53BP1 in cells treated with bortezomib, even at the concentration as high as 100 nM (I, Figure 2). In contrast, almost all of the cells treated with 5 Gy IR formed γH2AX, BRCA1 and 53BP1 foci that were evenly distributed in the nuclei, indicating DNA damage induction. Expression of γH2AX was observed in BRCA1-depleted cells treated with bortezomib, which was correlated with the expression of cPARP in Western blot. Here, γH2AX had perinuclear localization but did not form nuclear foci, thus reflecting ongoing apoptosis rather than DNA damage induction.

To further clarify if sensitization of BRCA1-depleted cells to proteasome inhibition involved DNA damage response, we depleted BARD1 and RNF8 in Hela cells followed by bortezomib treatment. BARD1 and BRCA1 form a constitutive heterodimer, which is required for the stability and E3 ligase activity of BRCA1 (Nishikawa et al. 2004; Xia et al. 2003). On the other hand, RNF8 is involved in the amplification of ubiquitin conjugates in response to DNA damage, but it is not required to maintain the function of BRCA1 (Kolas et al. 2007). Depletion of BARD1 in Hela cells increased their sensitivity to bortezomib, phenocopying depletion of BRCA1 (I, Figure 1h). In contrast, knockdown of RNF8 did not affect the sensitivity of cells to the proteasome inhibitor. These data strongly suggest that response to proteasome inhibitors involves a unique function of BRCA1 that is independent of DNA damage repair. This may also explain why BRCA2-depleted cells were not more sensitive to proteasome inhibitors.

4.2.3 Sensitization of BRCA1-depleted cells to proteasome inhibitors involves uncontrolled cell division caused by deregulated cell cycle checkpoints

Since the DNA repair function of BRCA1 was unlikely involved in the response to proteasome inhibitors, we sought to investigate whether its functions in the regulation of cell cycle checkpoints participated in the process. In BRCA1-depleted but not BRCA1-

proficient cells, bortezomib treatment led to increased phosphorylation of Rb (Ser807/811), as well as increased expression of cyclin B1 in a time-dependent manner (I, Figure 3b). Expression of cyclin B1 usually reaches a peak at the G2/M phase, during which it binds CDK1 to promote the transition of cells from G2 to M phase (Hochegger et al. 2008). Indeed, bortezomib treatment significantly induced accumulation of BRCA1-depleted cells at G2/M phase, while the proportion of cells at G1 phase was decreased, suggesting compromised G1 cell cycle checkpoint (I, Figure 3a). In contrast, distribution of G1 and G2/M phases was barely affected in BRCA1-proficient cells after bortezomib treatment.

Based on the observations above and the functions of Rb–E2F pathway in the regulation of cell cycle as well as apoptosis (van den Heuvel and Dyson 2008; Chau and Wang 2003), we hypothesized that inactivation of Rb might be responsible for the sensitization of BRCA1-depleted cells to proteasome inhibitors. Indeed, knockdown of Rb sensitized cells to bortezomib-induced apoptosis in a dose-dependent manner, reminiscent of the effect observed in BRCA1-depleted cells (I, Figure 3c). In addition, in BRCA1-depleted cells treated with bortezomib, the transcriptional activity of E2F1 was increased 2 fold in comparison with that in BRCA1-proficient or untreated cells (I, Figure 3d). Furthermore, the proteasome inhibitor-induced apoptosis in BRCA1-deficient cells was inhibited after depletion of E2F1 (I, Figure 3e). Taken together, these results demonstrate that in the absence of BRCA1, bortezomib treatment leads to the hyperphosphorylation and inactivation of Rb, which releases and activates its downstream transcription factor E2F1, leading to compromised G1 cell cycle checkpoint and accumulation of cells at G2/M phase accompanied by apoptosis.

One of the mechanisms responsible for cell death in G2/M phase involves mitotic catastrophe caused by defective G2/M checkpoint resulting in premature entry into cell division. Interestingly, a recent study identified several G2/M checkpoint regulators, e.g. cyclin B1 and CDC25C, as ubiquitin substrates of BRCA1 (Shabbeer et al. 2013). This raised a question as to whether sensitization of BRCA1-depleted cells to proteasome inhibitors could be due to unleashed cell division caused by increased expression of cyclin B1 after loss of BRCA1. In order to gauge the proportion of cells at mitosis, we measured the mitotic index by staining cells with a mitotic marker, phospho-histone H3 (Ser10). In control cells expressing functional BRCA1, bortezomib treatment significantly reduced the proportion of phospho-histone H3-positive cells, indicating a lower cell division rate consistent with a decreased level of cyclin B1 (I, Figure 3g). In contrast, the mitotic index of BRCA1-depleted cells remained high after bortezomib treatment, indicating uncontrolled cell division after loss of BRCA1. Taken together, our data indicate that proteasome inhibition induces compromised G1 and G2/M cell cycle checkpoints in BRCA1-depleted cells, leading to uncontrolled cell division and thus apoptotic cell death.

4.2.4 53BP1 depletion inhibits sensitization of BRCA1-deficient cells to proteasome inhibitors

Sensitization of BRCA1-depleted cells to proteasome inhibitors was validated in Hela and U2OS cell lines, which had even more striking effects than in MDA-MB-231 cells. A major difference between MDA-MB-231 and the other two cell lines is that MDA-MB-231 is p53 mutant, whereas the other two express wildtype p53. p53 regulates a wide spectrum of cellular activities, including transcriptional activation, cell cycle arrest and apoptosis induction, all of which contribute to its tumor suppressor function (Vousden and Prives 2009). Hence, we asked if the stronger sensitizing effects observed in Hela and U2OS were due to the roles of p53. Transfection of p53 siRNA markedly suppressed p53 activities as indicated by decreased expressions of both p53 and its downstream target p21. Surprisingly, in the absence of p53, bortezomib induced almost equal amount of cleaved PARP in BRCA1-depleted cells, compared with the control cells expressing functional p53 (I, Supplementary Figure S2). Thus, sensitization of BRCA1-depleted cells to proteasome inhibitors is p53 independent.

Recently, 53BP1 was found to functionally interact with BRCA1. *Brca1*-knockout mice are embryonic lethal due to dysfunctional homologous recombination (Cao et al. 2009). Knockout of *53bp1*, but not *p53*, rescues the embryonic lethality, indicating a specific role of 53bp1 in mediating cell death after loss of Brca1. Furthermore, loss of 53BP1 reverses the increased sensitivity of BRCA1-deficient cells to DNA-damaging agents, e.g. cisplatin and PARP inhibitors (Bunting et al. 2010). In order to see if 53BP1 plays a similar role in mediating response of BRCA1-depleted cells to proteasome inhibitors, we depleted both 53BP1 and BRCA1 with siRNAs in Hela cells, followed by treating the cells with bortezomib. Interestingly, knockdown of 53BP1 alleviated the apoptotic effect of BRCA1-depleted cells induced by bortezomib, as shown by decreased levels of cPARP (I, Figure 4c). In addition, knockdown of 53BP1 partially reversed the accumulation of BRCA1-depleted cells at G2/M phase after bortezomib treatment (I, Figure 4a), further confirming a causal role of G2/M dysregulation in sensitizing BRCA1-depleted cells to proteome inhibitors. Moreover, depletion of 53BP1 reactivated Rb in BRCA1-depleted cells treated with bortezomib, as indicated by its hypophosphorylation (I, Figure 4d), consistent with a slightly increased proportion of cells at G1 phase. On the other hand, knockdown of E2F1, which also inhibited apoptosis of BRCA1-depleted cells, did not affect the phosphorylation status of Rb, consistent with the fact that E2F1 is at the downstream of Rb. These results clearly demonstrate that 53BP1 plays a role in sensitizing BRCA1-depleted cells to proteasome inhibitors through cell cycle regulation.

Our data pointed out a role of BRCA1 in checkpoint regulation at both G1 and G2/M phases. Thus, loss of BRCA1 leads to the inactivation of Rb after bortezomib treatment,

which results in compromised G1 cell cycle checkpoint and accumulation of cells at G2/M phase. Proteasome inhibition also induces the expression of cyclin B1 in BRCA1-depleted cells, which leads to the uncontrolled cell division resulting in apoptotic cell death. We have also shown that sensitization of BRCA1-depleted cells to proteasome inhibitors can be partially inhibited by 53BP1 depletion, which rescues compromised G1 and G2/M cell cycle checkpoints. Combining these data together, it is conceivable that knockdown of 53BP1 in BRCA1-depleted cells would partially prevent uncontrolled cell division induced by bortezomib. Still, a key question here is how Rb is regulated by BRCA1 and 53BP1. It has been discovered long before that BRCA1 binds to hypophosphorylated Rb, which is required for sustaining Rb activity (Aprelikova et al. 1999). Consequently, it is likely that loss of BRCA1 releases Rb, which is then phosphorylated and inactivated by certain kinases during cell cycle progression. The link between 53BP1 and Rb is even more unclear, largely due to a lack of understanding on the roles of 53BP1 in cell cycle regulation. Only recently, it was found that 53BP1 can bind to Rb in a methylation-dependent manner, which is implicated in cell cycle regulation (Carr et al. 2014). Taken together, our data further establish the roles of BRCA1 in cell cycle regulation, loss of which sensitizes cells to proteasome inhibitors through deregulated Rb–E2F pathway.

4.3 Loss of BRCA1 promotes basal-like differentiation of mammary epithelial cells by sustaining ΔNp63 activity (Appendix II)

4.3.1 Loss of BRCA1 promotes the transition of mammary epithelial cells towards the basal lineage

BRCA1-mutant breast cancer usually belongs to the basal-like subtype, which is characterized by a triple-negative phenotype (Foulkes et al. 2004; Venkitaraman 2009). This raises a key question as to whether BRCA1 plays any roles in the regulation of cellular differentiation and, thus, its mutation restricts the development of cancer cells to a specific subtype. Moreover, basal-like breast tumors, including the BRCA1-mutant ones, usually have mutant p53, which has been shown to play a role in BRCA1-associated tumorigenesis (Xu et al. 2001; Badve et al. 2011). This brings a high possibility that BRCA1 mutations may regulate the differentiation of breast cancer cells through functional interaction with p53. In order to study the differentiation of luminal and basal mammary epithelial lineages, we established an *in vitro* model with freshly isolated mouse mammary epithelial cells (mMECs), which gradually switched the expression of lineage markers from luminal to basal types. Expression of keratin 18 (Krt18) was used as a luminal marker, while the basal-like phenotype was indicated by the expression of ΔNp63. With this model, we first found that p53 is required for the basal-like differentiation of mammary epithelial cells. Loss of p53 inhibited the transition of mammary epithelial cells from luminal to basal lineages,

accompanied by translocalization of ΔNp63 from nucleoplasms into the nucleoli, indicating its inactivation (II, Figure 1). In contrast, activation of p53 in luminal MCF-7 cells, either by MDM2 siRNA or by its inhibitor serdemetan, promoted the expression of ΔNp63, which was also translocated into the nucleoplasm (II, Figure 3c). These data firmly establish a role of p53 in the regulation of basal-like differentiation of mammary epithelial cells *in vitro*.

One of the effects resulted from BRCA1 loss is the induction of DNA damage response, which leads to activation of many genes, including p53. We hypothesized that loss of BRCA1 may induce basal-like differentiation of mammary epithelial cells due to p53 activation. In order to test this hypothesis *in vitro*, we depleted BRCA1 in MCF-7 cells by siRNA transfection, followed by measuring the expression of ΔNp63. Indeed, depletion of BRCA1 in MCF-7 cells resulted in the activation of p53, as well as ΔNp63, which was translocated from nucleoli into the nucleoplasm, indicating a transition from luminal to basal lineages (II, Figure 5a). In addition, inhibition of BRCA1 suppressed the expression of MDM2, and vice versa, suggesting their collaborating roles in regulating p53 activity (II, Fig. 5). These results suggest that loss of BRCA1 activates p53, which in turn induces ΔNp63 activation, and promotes the differentiation of mMECs from luminal to basal lineages.

4.3.2 BRCA1 antagonizes the activation of ΔNp63

Our results above reveal a p53-mediated link between loss of BRCA1 and activation of ΔNp63, which promotes the transition of mammary epithelial cells towards the basal lineage. However, almost all basal-like breast tumors, including BRCA1-mutant ones, have mutated p53, which raises a question as to how loss of BRCA1 leads to the development of basal-like breast cancer in the absence of functional p53. In order to further investigate the mechanisms underlying loss of BRCA1 and ΔNp63 activation, we utilized a basal-like cell line MCF-10A that is depleted for p53 (MCF-10A-p53$^{-/-}$). As inhibition of BRCA1 in the luminal-like MCF-7 cells leads to p53 activation and induction of the basal marker ΔNp63, we asked whether the opposite would be true for basal-like cells. ΔNp63 was highly expressed in the nucleoplasm of the basal-like MCF-10A cells, which have low expression of BRCA1 (II, Figure 6e). In contrast, MCF-10A cells with isogenic knockout of p53 (MCF-10A-p53$^{-/-}$) showed a decreased level of ΔNp63, which filled the nucleoli as well as the nucleoplasm. These cells also expressed high levels of BRCA1 and MDM2, suggesting antagonizing roles between the two proteins and ΔNp63. In addition, MCF-10A-p53$^{-/-}$ cells also expressed high levels of Krt18, indicating stronger luminal characters after p53 depletion. Interestingly, knockdown of BRCA1 in MCF-10A-p53$^{-/-}$ cells rescued the expression of ΔNp63, suggesting a basal-like transition after loss of BRCA1 despite the absence of p53 (II, Figure 7). Taken together, these data indicate that the activity of ΔNp63

is antagonized by BRCA1. Consequently, loss of BRCA1 leads to the activation of ΔNp63, which then promotes the differentiation of mammary epithelial cells towards the basal lineage even in the absence of p53.

Our results suggest a potential mechanism of BRCA1 in regulating differentiation of mammary epithelial cells (II, Figure 8). Almost all BRCA1-mutant breast tumors are mutant with p53. Surprisingly, our results showed that loss of p53 promotes the luminal phenotype and impairs basal-like differentiation through inhibition of ΔNp63. However, we observed that if BRCA1 was depleted together with p53, the basal-like phenotype was rescued. Currently, it remains unknown as to how functional BRCA1 antagonizes ΔNp63 activity, but ubiquitin-mediated proteasomal degradation might be a possibility, as BRCA1 possesses ubiquitin E3 ligase activity. This may contribute to the sustained activity of ΔNp63 in the BRCA1-mutant, p53-depleted cells. It has been recently found that the basal-like breast cancers originate from luminal progenitor cells. Together with our data, this may help to explain how loss of BRCA1 initiates the development of basal-like breast cancer.

5. Concluding remarks

Here in this study, we aimed to better understand the biology of breast cancers associated BRCA1 mutations in order to develop potential therapeutics for their treatment. For these purposes, we first characterized four BRCA1-mutant breast cancer cell lines as surrogates of breast tumors caused by BRCA1 dysfunction. Despite the essential roles of BRCA1 in DNA damage response and repair, the BRCA1-mutant cell lines are generally resistant to several DNA-damaging agents. The resistant phenotypes of different cell lines might be due to different reasons. SUM149PT cells could form RAD51 foci after ionizing radiation, indicating functional homologous recombination. Other cells with BRCA1 mutations may survive damaging insults by mutating p53 or through other mechanisms yet to be characterized. Additionally, our results from the high-throughput screen and proteome profiling assay revealed that the BRCA1-mutant cell lines are more resistant to MEK inhibitors that the BRCA1-wildtype cells.

From a high-throughput chemical compound screen, we found that proteasome inhibitors bortezomib and carfilzomib are able to selectively kill BRCA1-deficient cells. Further mechanistic studies demonstrated that the sensitizing effect is not due to dysfunctional DNA damage repair after loss of BRCA1; rather it is caused by compromised G1 and G2/M cell cycle checkpoints resulting in uncontrolled mitosis of BRCA1-deficient cells. Furthermore, sensitization of BRCA1-deficient cells to bortezomib is independent of p53 but depends on 53BP1. Bortezomib has been approved for the treatment of multiple myeloma, demonstrating its safety in clinics. These findings raise a high potential of targeting proteasome for the treatment of BRCA1-mutant breast cancer, especially considering the facts that BRCA1-mutant tumors are almost always p53 mutant and many are resistant to DNA-damaging agents.

It has been observed that BRCA1 mutations usually lead to breast tumors of the basal-like subtype, suggesting a function of BRCA1 in regulating mammary cellular differentiation. Here we find that loss of BRCA1 leads to stabilization and activation of ΔNp63, which promotes the transition of mouse mammary epithelial cells (mMECs) from luminal to basal lineages. This finding provides a preliminary mechanism that suggests a causal role of BRCA1 loss in initiating basal-like breast cancer.

Acknowledgements

At the end the writing, looking back at the past five years, I must say I couldn't have finished this work by myself. I would like to express my gratitude to all of the people who have helped me to make the work as described in this book, which hopefully would be printed and preserved forever.

First of all, I would like to thank my supervisor, Dr. Sergey Kuznetsov, for giving me the opportunity to come to Finland and start my PhD here. Without that, nothing mentioned in the book in the past five years could happen. I also would like to thank him for the supervision of the projects, from which I have learned a lot.

I would like to express my sincere thanks to my thesis committee members, Prof. Marikki Laiho and Dr. Juha Klefström. They have given me many useful suggestions in my projects during thesis committee meetings. I admire them for their scientific knowledge and thank them for their kind help given to me. I have also learned a lot of knowledge in science from communications with them. I would also express my gratitude to Dr. Gretchen Repasky as a coordinator at FIMM. Without your kind help, I even couldn't reach this step. Thank you very much, Gretchen!

This work was mainly carried out at FIMM. I appreciate the state-of-the-art infrastructure established by the Institute as well as the expertise of the researchers around, which helped to make great science possible. Our collaborators in Finland and the Netherlands have promoted the quality of our research as well, so thank you very much! Also, I would like to thank the Integrative Life Science Doctoral Program (ILS) for their financial support, which provided me a decent life in Helsinki.

I would like to thank everyone around me at FIMM, people from the three groups in E Wing, Manu, Pauliina, Daria, Emmy, Jenni, Anna, Katja, Ashwini, Sarang, Virva, Maria and Oxana. We have spent a lot of time together. Special thanks to Manu who introduced me to the lab when I first came here as a new person; and to Pauliina who allowed me to participate in her project, which has helped promote me to this stage. I would like to thank Dennis, Teijo, Khalid, Dat and Susanne for our friendships. Many staff and students in FIMM have given me help at times. I will remember the happy times with you in the future. Thank you all!

I sincerely thank Professor Kirill Alexandrov from the Institute for Molecular Bioscience (IMB), University of Queensland, who brought me abroad six years ago.

Finally, I would thank my family, the ones who I love and love me, for their tolerance, support and pride of me, even though they don't really know what I was doing. No matter where you are and where I am, you are the only reason of my whole life!

References

Ahn JY, Li X, Davis HL, Canman CE (2002) Phosphorylation of threonine 68 promotes oligomerization and autophosphorylation of the Chk2 protein kinase via the forkhead-associated domain. The Journal of Biological Chemistry 277: 19389-19395

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proceedings of the National Academy of Sciences of the United States of America 100: 3983-3988

Allan AL, Vantyghem SA, Tuck AB, Chambers AF (2007). Tumor dormancy and cancer stem cells: implications for the biology and treatment of breast cancer metastasis. Breast Dis. 26:87–98.

Alli E, Sharma VB, Hartman AR, Lin PS, McPherson L, Ford JM (2011) Enhanced sensitivity to cisplatin and gemcitabine in Brca1-deficient murine mammary epithelial cells. BMC Pharmacology 11: 7

Almeida KH, Sobol RW (2007) A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification. DNA Repair 6: 695-711

Al-Minawi AZ, Lee YF, Hakansson D, Johansson F, Lundin C, Saleh-Gohari N, Schultz N, Jenssen D, Bryant HE, Meuth M, Hinz JM, Helleday T (2009) The ERCC1/XPF endonuclease is required for completion of homologous recombination at DNA replication forks stalled by inter-strand cross-links. Nucleic Acids Research 37: 6400-6413

Alvarado M, Ozanne E, Esserman L (2011) Overdiagnosis and Overtreatment of Breast Cancer. In American Society of Clinical Oncology educational book/ASCO. American Society of Clinical Oncology. Meeting, Vol. 32, pp e40-e45

Antoniou A, Easton D (2006) Models of genetic susceptibility to breast cancer. Oncogene 25: 5898-5905

Antoniou A, Pharoah P, McMullan G, Day N, Stratton M, Peto J, Ponder B, Easton D (2002) A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. British Journal of Cancer 86: 76-83

Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tulinius H, Thorlacius S, Eerola H, Nevanlinna H, Syrjakoski K, Kallioniemi OP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG, Easton DF (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. American Journal of Human Genetics 72: 1117-1130

Apostolou P, Fostira F (2013) Hereditary breast cancer: the era of new susceptibility genes. BioMed Research International 2013: 747318.

Aprelikova ON, Fang BS, Meissner EG, Cotter S, Campbell M, Kuthiala A, Bessho M, Jensen RA, Liu ET (1999) BRCA1-associated growth arrest is RB-dependent. Proceedings of the National Academy of Sciences of the United States of America 96: 11866-11871

Audeh M, Penson R, Friedlander M, Powell B, Bell-McGuinn K, Scott C, Weitzel J, Carmichael J, Tutt A (2009) Phase II trial of the oral PARP inhibitor olaparib (AZD2281) in BRCA-deficient advanced ovarian cancer. ASCO Annual Meeting. Journal of Clinical Oncology 27: Abstract 5500

August DA, Kearney T (2000) Cystosarcoma phyllodes: mastectomy, lumpectomy, or lumpectomy plus irradiation. Surgical Oncology 9: 49-52

Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR (2011) Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Modern Pathology 24: 157-167

Baer R, Ludwig T (2002) The BRCA1/BARD1 heterodimer, a tumor suppressor complex with ubiquitin E3 ligase activity. Current Opinion in Genetics & Development 12: 86-91

Bal A, Verma S, Joshi K, Singla A, Thakur R, Arora S, Singh G (2012) BRCA1-methylated sporadic breast cancers are BRCA-like in showing a basal phenotype and absence of ER expression. Virchows Archiv 461: 305-312

Banin S, Moyal L, Shieh S, Taya Y, Anderson CW, Chessa L, Smorodinsky NI, Prives C, Reiss Y, Shiloh Y, Ziv Y (1998) Enhanced phosphorylation of p53 by ATM in response to DNA damage. Science 281: 1674-1677

Bartek J, Lukas C, Lukas J (2004) Checking on DNA damage in S phase. Nature Reviews Molecular Cell Biology 5: 792-804

Bartek J, Lukas J (2001) Mammalian G1- and S-phase checkpoints in response to DNA damage. Current Opinion in Cell Biology 13: 738-747

Barton S, Swanton C (2011) Recent developments in treatment stratification for metastatic breast cancer. Drugs 12: 2099-2113

Baumann P, Benson FE, West SC (1996) Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. Cell 87: 757-766

Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S, Goodarzi AA, Krempler A, Jeggo PA, Löbrich M (2009) ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. The EMBO Journal 28: 3413-3427

Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK (2000) The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. The Journal of Biological Chemistry 275: 23899-23903

Bianco PR, Tracy RB, Kowalczykowski SC (1998) DNA strand exchange proteins: a biochemical and physical comparison. Front Biosci 3: D570-D603

Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, Pommier Y (2008) γH2AX and cancer. Nature Reviews Cancer 8: 957-967

Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, Hiddingh S, Thanasoula M, Kulkarni A, Yang Q, Haffty BG, Tommiska J, Blomqvist C, Drapkin R, Adams DJ, Nevanlinna H, Bartek J, Tarsounas M, Ganesan S, Jonkers J (2010)

53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nature Structural & Molecular Biology 17: 688-695

Bouwman P, Jonkers J (2012) The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nature Reviews Cancer 12: 587-598

Bouwman P, van der Gulden H, van der Heijden I, Drost R, Klijn CN, Prasetyanti P, Pieterse M, Wientjens E, Seibler J, Hogervorst FB, Jonkers J (2013) A high-throughput functional complementation assay for classification of BRCA1 missense variants. Cancer Discovery 3: 1142-1155

Boyd J, Sonoda Y, Federici MG, Bogomolniy F, Rhei E, Maresco DL, Saigo PE, Almadrones LA, Barakat RR, Brown CL, Chi DS, Curtin JP, Poynor EA, Hoskins WJ (2000) Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. JAMA. 283: 2260-2265

Braithwaite EK, Prasad R, Shock DD, Hou EW, Beard WA, Wilson SH (2005) DNA polymerase λ mediates a back-up base excision repair activity in extracts of mouse embryonic fibroblasts. Journal of Biological Chemistry 280: 18469-18475

Brem R, Hall J (2005) XRCC1 is required for DNA single-strand break repair in human cells. Nucleic Acids Research 33: 2512-2520

Bruhn C, Zhou Z-W, Ai H, Wang Z-Q (2014) The Essential Function of the MRN Complex in the Resolution of Endogenous Replication Intermediates. Cell Reports 6: 182-195

Brulikova L, Hlavac J, Hradil P (2012) DNA interstrand cross-linking agents and their chemotherapeutic potential. Current Medicinal Chemistry 19: 364-385

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase. Nature 434: 913-917

Buisson R, Dion-Cote AM, Coulombe Y, Launay H, Cai H, Stasiak AZ, Stasiak A, Xia B, Masson JY (2010) Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. Nature Structural & Molecular Biology 17: 1247-1254

Bunting SF, Callen E, Wong N, Chen HT, Polato F, Gunn A, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cao L, Xu X, Deng CX, Finkel T, Nussenzweig M, Stark JM, Nussenzweig A (2010) 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 141: 243-254

Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ (2001) ATM phosphorylates histone H2AX in response to DNA double-strand breaks. Journal of Biological Chemistry 276: 42462-42467

Cai Z, Chehab NH, Pavletich NP (2009) Structure and activation mechanism of the CHK2 DNA damage checkpoint kinase. Molecular Cell 35: 818-829

Caldecott KW (2008) Single-strand break repair and genetic disease. Nature Reviews Genetics 9: 619-631

Campbell LL, Polyak K (2007) Breast tumor heterogeneity: cancer stem cells or clonal evolution? Cell Cycle 6: 2332-2338

Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490: 61-70

Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, Appella E, Kastan MB, Siliciano JD (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science 281: 1677-1679

Cao L, Xu X, Bunting SF, Liu J, Wang RH, Cao LL, Wu JJ, Peng TN, Chen J, Nussenzweig A, Deng CX, Finkel T (2009) A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency. Molecular Cell 35: 534-541

Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S (2006) Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 295: 2492-2502

Carr SM, Munro S, Zalmas LP, Fedorov O, Johansson C, Krojer T, Sagum CA, Bedford MT, Oppermann U, La Thangue NB (2014) Lysine methylation-dependent binding of 53BP1 to the pRb tumor suppressor. Proceedings of the National Academy of Sciences of the United States of America 111: 11341-11346

Carrassa L, Broggini M, Erba E, Damia G (2004) Report Chk1, but not Chk2, is Involved in the Cellular Response to DNA Damaging Agents. Cell Cycle 3: 1177-1181

Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A (2003) Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nature Cell Biology 5: 675-679

Chapman JR, Jackson SP (2008) Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. EMBO Reports 9: 795-801

Chapman JR, Sossick AJ, Boulton SJ, Jackson SP (2012) BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. Journal of Cell Science 125: 3529-3534

Chau BN, Wang JY (2003) Coordinated regulation of life and death by RB. Nature Reviews Cancer 3: 130-138

Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS (2009) Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. Journal of the National Cancer Institute 101: 736-50

Chehab NH, Malikzay A, Appel M, Halazonetis TD (2000) Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. Genes & Development 14: 278-288

CHEK2 Breast Cancer Case-Control Consortium (2004) CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. American Journal of Human Genetics 74: 1175-1182

Chen A, Kleiman FE, Manley JL, Ouchi T, Pan Z-Q (2002) Autoubiquitination of the BRCA1 ⁃BARD1 RING ubiquitin ligase. Journal of Biological Chemistry 277: 22085-22092

Chen H, Lisby M, Symington LS (2013) RPA coordinates DNA end resection and prevents formation of DNA hairpins. Molecular Cell 50: 589-600

Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Couch FJ, Weber BL, Ashley T, Livingston DM, Scully R (1998) Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Molecular Cell 2: 317-328

Chen L, Gilkes DM, Pan Y, Lane WS, Chen J (2005) ATM and Chk2-dependent phosphorylation of MDMX contribute to p53 activation after DNA damage. The EMBO Journal 24: 3411-3422

Chen L, Nievera CJ, Lee AY-L, Wu X (2008) Cell cycle-dependent complex formation of BRCA1 · CtIP · MRN is important for DNA double-strand break repair. Journal of Biological Chemistry 283: 7713-7720

Choi YH, Yu AM (2014) ABC transporters in multidrug resistance and pharmacokinetics, and strategies for drug development. Current Pharmaceutical Design 20: 793-807

Cleare M, Hydes P, Hepburn D, Malerbi B, Prestayko A, Crooke S, Carter S (1980) Cisplatin, Current Status and New Developments. Academic Press, New York: 149

Collis SJ, DeWeese TL, Jeggo PA, Parker AR (2004) The life and death of DNA-PK. Oncogene 24: 949-961

Consortium CBCC-C (2004) CHEK2* 1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. American Journal of Human Genetics 74: 1175-1182

Cooper GM, Hausman RE (2000) The cell: a Molecular Approach. 2nd Edition. Sinauer Associates Sunderland.

Cortez D, Wang Y, Qin J, Elledge SJ (1999) Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science 286: 1162-1166

Costa R, Chigancas V, da Silva Galhardo R, Carvalho H, Menck CF (2003) The eukaryotic nucleotide excision repair pathway. Biochimie 85: 1083-1099

Couch FJ, Weber BL (1996) Mutations and Polymorphisms in the familial early-onset breast cancer (BRCA1) gene. Human Mutation 8: 8-18

Cruz-García A, López-Saavedra A, Huertas P (2014) BRCA1 accelerates CtIP-mediated DNA-end resection. Cell Reports 9: 451-459

Cui X, Yu Y, Gupta S, Cho Y-M, Lees-Miller SP, Meek K (2005) Autophosphorylation of DNA-dependent protein kinase regulates DNA end processing and may also alter double-strand break repair pathway choice. Molecular and Cellular Biology 25: 10842-10852

Curtin NJ (2012) DNA repair dysregulation from cancer driver to therapeutic target. Nature Reviews Cancer 12: 801-817

Dalerba P, Cho RW, Clarke MF (2007) Cancer stem cells: models and concepts. Annual Review of Medicine 58: 267-284

Davies AA, Masson JY, McIlwraith MJ, Stasiak AZ, Stasiak A, Venkitaraman AR, West SC (2001) Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. Molecular Cell 7: 273-282

Deans AJ, West SC (2011) DNA interstrand crosslink repair and cancer. Nature Reviews Cancer 11: 467-480

Delaney G, Jacob S, Featherstone C, Barton M (2005) The role of radiotherapy in cancer treatment. Cancer 104: 1129-1137

Deng C (2006) BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. Nucleic Acids Research 34: 1416-1426

DeVita VT, Chu E (2008) A History of cancer chemotherapy. Cancer Research 68: 8643-8653

Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, Schurra C, Garre M, Nuciforo PG, Bensimon A (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 444: 638-642

Dietlein F, Thelen L, Reinhardt HC (2014) Cancer-specific defects in DNA repair pathways as targets for personalized therapeutic approaches. Trends in Genetics 30: 326-339

DiPaola RS (2002) To arrest or not to G(2)-M Cell-cycle arrest. Clinical Cancer Research. 8: 3311-3314

DiTullio RA, Mochan TA, Venere M, Bartkova J, Sehested M, Bartek J, Halazonetis TD (2002) 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. Nature Cell Biology 4: 998-1002

Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, Ellenberg J, Panier S, Durocher D, Bartek J, Lukas J, Lukas C (2009) RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell 136: 435-446

Dolan ME, Moschel RC, Pegg AE (1990) Depletion of mammalian O6-alkylguanine-DNA alkyltransferase activity by O6-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents. Proceedings of the National Academy of Sciences of the United States of America 87: 5368-5372

Dong Y, Hakimi M-A, Chen X, Kumaraswamy E, Cooch NS, Godwin AK, Shiekhattar R (2003) Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. Molecular Cell 12: 1087-1099

Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS (2003) In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes & Development 17: 1253-1270

Drew Y, Jones A, Hall G, Jayson GC, Highley M, Rea D, Glasspool RM, Halford SR, Crosswell G, Colebrook S, Boddy AV, Curtin NJ, Plummer ER (2011). Phase II trial of the poly(ADP-ribose) polymerase (PARP) inhibitor AG-014699 in BRCA 1 and 2–mutated, advanced ovarian and/or locally advanced or metastatic breast cancer. ASCO Annual Meeting. Journal of Clinical Oncology 29: Abstract 3104

Drouet J, Frit P, Delteil C, de Villartay J-P, Salles B, Calsou P (2006) Interplay between Ku, Artemis, and the DNA-dependent protein kinase catalytic subunit at DNA ends. Journal of Biological Chemistry 281: 27784-27793

Drukker C, Bueno-de-Mesquita J, Retel V, Harten W, Tinteren H, Wesseling J, Roumen R, Knauer M, Veer L, Sonke G (2013) A prospective evaluation of a breast cancer

prognosis signature in the observational RASTER study. International Journal of Cancer 133: 929-936

Eakin CM, MacCoss MJ, Finney GL, Klevit RE (2007) Estrogen receptor α is a putative substrate for the BRCA1 ubiquitin ligase. Proceedings of the National Academy of Sciences of the United States of America 104: 5794-5799

Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447: 1087-1093

Eckert KA, Hile SE (2009) Every microsatellite is different: Intrinsic DNA features dictate mutagenesis of common microsatellites present in the human genome. Molecular Carcinogenesis 48: 379-388

Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, Hankinson SE (2006) Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. Journal of the National Cancer Institute 98: 1406-1415

Ellisen M, Leif W, Haber M, Daniel A (1998) Hereditary breast cancer. Annual Review of Medicine 49: 425-436

Elstrodt F, Hollestelle A, Nagel JH, Gorin M, Wasielewski M, van den Ouweland A, Merajver SD, Ethier SP, Schutte M (2006) BRCA1 mutation analysis of 41 human breast cancer cell lines reveals three new deleterious mutants. Cancer Research 66: 41-45

Euhus DM, Diaz J (2015) Breast Cancer Prevention. The Breast Journal 1: 76-81

Fabbro M, Savage K, Hobson K, Deans AJ, Powell SN, McArthur GA, Khanna KK (2004) BRCA1-BARD1 complexes are required for p53Ser-15 phosphorylation and a G1/S arrest following ionizing radiation-induced DNA damage. The Journal of Biological Chemistry 279: 31251-31258

Falck J, Coates J, Jackson SP (2005) Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature 434: 605-611

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434: 917-921

Feeley LP, Mulligan AM, Pinnaduwage D, Bull SB, Andrulis IL (2013) Distinguishing luminal breast cancer subtypes by Ki67, progesterone receptor or TP53 status provides prognostic information. Mod Pathology 27: 554-561

Feng L, Fong KW, Wang J, Wang W, Chen J (2013) RIF1 counteracts BRCA1-mediated end resection during DNA repair. The Journal of Biological Chemistry 288: 11135-11143

Feng L, Huang J, Chen J (2009) MERIT40 facilitates BRCA1 localization and DNA damage repair. Genes & Development 23: 719–728

Fiets W, Van Helvoirt R, Nortier J, Van der Tweel I, Struikmans H (2003) Acute toxicity of concurrent adjuvant radiotherapy and chemotherapy (CMF or AC) in breast cancer patients: a prospective, comparative, non-randomised study. European Journal of Cancer 39: 1081-1088

Foekens JA, Atkins D, Zhang Y, Sweep FC, Harbeck N, Paradiso A, Cufer T, Sieuwerts AM, Talantov D, Span PN (2006) Multicenter validation of a gene expression–based prognostic signature in lymph node–negative primary breast cancer. Journal of Clinical Oncology 24: 1665-1671

Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ (2009) Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. New England Journal of Medicine 361: 123-134

Fong PC, Yap TA, Boss DS, Carden CP, Mergui-Roelvink M, Gourley C, De Greve J, Lubinski J, Shanley S, Messiou C, A'Hern R, Tutt A, Ashworth A, Stone J, Carmichael J, Schellens JH, de Bono JS, Kaye SB (2010) Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. Journal of Clinical Oncology 28: 2512-2519

Foulkes WD (2004) BRCA1 functions as a breast stem cell regulator. Journal of Medical Genetics 41: 1-5

Foulkes WD (2006) BRCA1 and BRCA2: chemosensitivity, treatment outcomes and prognosis. Familial Cancer 5: 135-142

Foulkes WD, Metcalfe K, Sun P, Hanna WM, Lynch HT, Ghadirian P, Tung N, Olopade OI, Weber BL, McLennan J (2004) Estrogen receptor status in BRCA1-and BRCA2-related breast cancer: the influence of age, grade, and histological type. Clinical Cancer Research 10: 2029-2034

Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. New England Journal of Medicine 363: 1938-1948

Fousteri M, Mullenders LH (2008) Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Research 18: 73-84

Freitas AA, de Magalhaes JP (2011) A review and appraisal of the DNA damage theory of ageing. Mutation Research/Reviews in Mutation Research 728: 12-22

Frizzell KM, Kraus WL (2009) PARP inhibitors and the treatment of breast cancer: beyond BRCA1/2? Breast Cancer Research 11: 111

Fu D, Calvo JA, Samson LD (2012) Balancing repair and tolerance of DNA damage caused by alkylating agents. Nature Reviews Cancer 12: 104-120

Gajria D, Chandarlapaty S (2011) HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. Expert Review of Anticancer Therapy 11: 263-275

Garcia-Closas M, Chanock S (2008) Genetic susceptibility loci for breast cancer by estrogen receptor status. Clinical Cancer Research 14: 8000-8009

Gates KS (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. Chemical Research in Toxicology 22: 1747-1760

Gillet LC, Schärer OD (2006) Molecular mechanisms of mammalian global genome nucleotide excision repair. Chemical Reviews 106: 253-276

Gilman A (1963) The initial clinical trial of nitrogen mustard. The American Journal of Surgery 105: 574-578

Gorgoulis VG, Vassiliou L-VF, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, Venere M, DiTullio RA, Kastrinakis NG, Levy B (2005) Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434: 907-913

Gravel S, Chapman JR, Magill C, Jackson SP (2008) DNA helicases Sgs1 and BLM promote DNA double-strand break resection. Genes & Development 22: 2767-2772

Greenberg RA, Sobhian B, Pathania S, Cantor SB, Nakatani Y, Livingston DM (2006) Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. Genes & Development 20: 34-46

Gudmundsdottir K, Ashworth A (2006) The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. Oncogene 25: 5864-5874

Gusterson BA, Ross DT, Heath VJ, Stein T (2005) Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer. Breast Cancer Research 7: 143-148

Habel LA, Shak S, Jacobs MK, Capra A, Alexander C, Pho M, Baker J, Walker M, Watson D, Hackett J (2006) A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. Breast Cancer Research 8: R25

Halazonetis TD, Gorgoulis VG, Bartek J (2008) An oncogene-induced DNA damage model for cancer development. Science 319: 1352-1355

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King M-C (1990) Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250: 1684-1689

Harrington JM, Kolodner RD (2007) Saccharomyces cerevisiae Msh2-Msh3 acts in repair of base-base mispairs. Molecular and Cellular Biology 27: 6546-6554

Harris TJ, McCormick F (2010) The molecular pathology of cancer. Nature Reviews Clinical Oncology 7: 251-265

Hegi ME, Diserens A-C, Gorlia T, Hamou M-F, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. New England Journal of Medicine 352: 997-1003

Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA (2008) DNA repair pathways as targets for cancer therapy. Nature Reviews Cancer 8: 193-204

Herschkowitz JI, Simin K, Weigman VJ, Mikaelian I, Usary J, Hu Z, Rasmussen KE, Jones LP, Assefnia S, Chandrasekharan S (2007) Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biology 8: R76

Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AI, Reaper PM, Jackson SP, Curtin NJ, Smith GC (2004) Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Research 64: 9152-9159

Hill VK, Gartner JJ, Samuels Y, Goldstein AM (2013) The genetics of melanoma: recent advances. Annual Review of Genomics and Human Genetics 14: 257-279

Hochegger H, Dejsuphong D, Fukushima T, Morrison C, Sonoda E, Schreiber V, Zhao GY, Saberi A, Masutani M, Adachi N (2006) Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells. The EMBO Journal 25: 1305-1314

Hochegger H, Takeda S, Hunt T (2008) Cyclin-dependent kinases and cell-cycle transitions: does one fit all? Nature Reviews Molecular Cell Biology 9: 910-916

Hoeijmakers JH (2009) DNA damage, aging, and cancer. New England Journal of Medicine 361: 1475-1485

Hosoya N, Miyagawa K (2014) Targeting DNA damage response in cancer therapy. Cancer Science 105: 370-388

Hsu NC, Huang YF, Yokoyama KK, Chu PY, Chen FM, Hou MF (2013) Methylation of BRCA1 promoter region is associated with unfavorable prognosis in women with early-stage breast cancer. PloS One 8: e56256

Hu J, Sun L, Shen F, Chen Y, Hua Y, Liu Y, Zhang M, Hu Y, Wang Q, Xu W, Sun F, Ji J, Murray JM, Carr AM, Kong D (2012) The intra-S phase checkpoint targets Dna2 to prevent stalled replication forks from reversing. Cell 149: 1221-1232

Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J (2007) RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell 131: 901-914

Huen MS, Sy SM, Chen J (2010) BRCA1 and its toolbox for the maintenance of genome integrity. Nature Reviews Molecular Cell Biology 11: 138-148

Huertas P, Jackson SP (2009) Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. Journal of Biological Chemistry 284: 9558–9565

Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors (2005) Nature Reviews Cancer 5: 341–354.

Ichikawa M, Nakane H, Marra G, Corti C, Jiricny J, Fitch M, Ford JM, Ikejima M, Shimada T, Yoshino M (2000) Decreased UV sensitivity, mismatch repair activity and abnormal cell cycle checkpoints in skin cancer cell lines derived from UVB-irradiated XPA-deficient mice. Mutation Research/DNA Repair 459: 285-298

Ignatiadis M, Sotiriou C (2013) Luminal breast cancer: from biology to treatment. Nature Reviews Clinical Oncology 10: 494-506

Ikura T, Tashiro S, Kakino A, Shima H, Jacob N, Amunugama R, Yoder K, Izumi S, Kuraoka I, Tanaka K (2007) DNA damage-dependent acetylation and ubiquitination of H2AX enhances chromatin dynamics. Molecular and Cellular Biology 27: 7028-7040

Inbar-Rozensal D, Castiel A, Visochek L, Castel D, Dantzer F, Izraeli S, Cohen-Armon M (2009) A selective eradication of human nonhereditary breast cancer cells by phenanthridine-derived polyADP-ribose polymerase inhibitors. Breast Cancer Research 11: R78

Isakoff BO SJ, Tung NM, Gelman RS, Giranda VL, Bernhard KM, Habin KR, Ellisen LW, Winer EP, Goss PE (2010) A phase II trial of the PARP inhibitor veliparib (ABT888)

and temozolomide for metastatic breast cancer. Journal of Clinical Oncology. ASCO Annual Meeting Abstracts. 2010: 1019

Izawa N, Wu W, Sato K, Nishikawa H, Kato A, Boku N, Itoh F, Ohta T (2011) HERC2 Interacts with Claspin and regulates DNA origin firing and replication fork progression. Cancer Research 71: 5621-5625

Jackson SP, Bartek J (2009) The DNA-damage response in human biology and disease. Nature 461: 1071-1078

Jacobs AL, Schär P (2012) DNA glycosylases: in DNA repair and beyond. Chromosoma 121: 1-20

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA: A Cancer Journal for Clinicians 61: 69-90

Jiang G, Wei Z-P, Pei D-S, Xin Y, Liu Y-Q, Zheng J-N (2011) A novel approach to overcome temozolomide resistance in glioma and melanoma: inactivation of MGMT by gene therapy. Biochemical and Biophysical Research Communications 406: 311-314

Johnson N, Li Y-C, Walton ZE, Cheng KA, Li D, Rodig SJ, Moreau LA, Unitt C, Bronson RT, Thomas HD (2011) Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. Nature Medicine 17: 875-882

Joukov V, Chen J, Fox EA, Green JB, Livingston DM (2001) Functional communication between endogenous BRCA1 and its partner, BARD1, during Xenopus laevis development. Proceedings of the National Academy of Sciences of the United States of America 98: 12078-12083

Jovanovic M, Dynan WS (2006) Terminal DNA structure and ATP influence binding parameters of the DNA-dependent protein kinase at an early step prior to DNA synapsis. Nucleic Acids Research 34: 1112-1120

Jung D, Giallourakis C, Mostoslavsky R, Alt FW (2006) Mechanism and control of V (D) J recombination at the immunoglobulin heavy chain locus. Annual Review of Immunology 24: 541-570

Kadyrov FA, Dzantiev L, Constantin N, Modrich P (2006) Endonucleolytic function of MutLα in human mismatch repair. Cell 126: 297-308

Kaelin WG (2005) The concept of synthetic lethality in the context of anticancer therapy. Nature Reviews Cancer 5: 689-698

Kakarougkas A, Ismail A, Klement K, Goodarzi AA, Conrad S, Freire R, Shibata A, Lobrich M, Jeggo PA (2013) Opposing roles for 53BP1 during homologous recombination. Nucleic Acids Research 41: 9719-9731

Kapp AV, Jeffrey SS, Langerød A, Børresen-Dale A-L, Han W, Noh D-Y, Bukholm IR, Nicolau M, Brown PO, Tibshirani R (2006) Discovery and validation of breast cancer subtypes. BMC Genomics 7: 231

Kastan MB, Bartek J (2004) Cell-cycle checkpoints and cancer. Nature 432: 316-323

Kat A, Thilly WG, Fang W-H, Longley MJ, Li G-M, Modrich P (1993) An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. Proceedings of the National Academy of Sciences of the United States of America 90: 6424-6428

Kaye SB (1998) New antimetabolites in cancer chemotherapy and their clinical impact. British Journal of Cancer 78: 1-7

Kaye SB, Lubinski J, Matulonis U, Ang JE, Gourley C, Karlan BY, Amnon A, Bell-McGuinn KM, Chen LM, Friedlander M, Safra T, Vergote I, Wickens M, Lowe ES, Carmichael J, Kaufman B (2012) Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer. Journal of Clinical Oncology 30: 372-379

Keegan KS, Holtzman DA, Plug AW, Christenson ER, Brainerd EE, Flaggs G, Bentley NJ, Taylor EM, Meyn MS, Moss SB (1996) The Atr and Atm protein kinases associate with different sites along meiotically pairing chromosomes. Genes & Development 10: 2423-2437

Kehe K, Balszuweit F, Emmler J, Kreppel H, Jochum M, Thiermann H (2008) Sulfur mustard research—strategies for the development of improved medical therapy. Eplasty 8: e32

Kim H, Chen J, Yu X (2007) Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science 316: 1202–1205

Kim SS, Cao L, Li C, Xu X, Huber LJ, Chodosh LA, Deng CX (2004) Uterus hyperplasia and increased carcinogen-induced tumorigenesis in mice carrying a targeted mutation of the Chk2 phosphorylation site in Brca1. Molecular and Cellular Biology 24: 9498-9507

King MC, Marks JH, Mandell JB (2003) Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 302: 643-646

Kinsella AR, Smith D, Pickard M (1997) Resistance to chemotherapeutic antimetabolites: a function of salvage pathway involvement and cellular response to DNA damage. British Journal of Cancer 75: 935-945

Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB (2004) Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes & Development 18: 1423-1438

Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, Pelletier L, Jackson SP, Durocher D (2007) Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science 318: 1637-1640

Kondo N, Takahashi A, Ono K, Ohnishi T (2010) DNA damage induced by alkylating agents and repair pathways. Journal of Nucleic Acids 2010: 543531

Koonin EV, Altschul SF, Bork P (1996) … Functional motifs…. Nature Genetics 13: 266-268

Kraemer KH, Patronas NJ, Schiffmann R, Brooks BP, Tamura D, DiGiovanna JJ (2007) Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: a complex genotype–phenotype relationship. Neuroscience 145: 1388-1396

Kramer A, Mailand N, Lukas C, Syljuasen RG, Wilkinson CJ, Nigg EA, Bartek J, Lukas J (2004) Centrosome-associated Chk1 prevents premature activation of cyclin-B-Cdk1 kinase. Nature Cell Biology 6: 884-891

Kunkel TA, Erie DA (2005) DNA mismatch repair. Annual Review of Biochemistry 74: 681-710

Kwei KA, Kung Y, Salari K, Holcomb IN, Pollack JR (2010) Genomic instability in breast cancer: pathogenesis and clinical implications. Molecular Oncology 4: 255-66

Lane TF, Deng C, Elson A, Lyu MS, Kozak CA, Leder P (1995) Expression of Brca1 is associated with terminal differentiation of ectodermally and mesodermally derived tissues in mice. Genes & Development 9: 2712-2722

Lavin MF (2004) The Mre11 complex and ATM: a two-way functional interaction in recognising and signaling DNA double strand breaks. DNA Repair (Amst) 3: 1515-1520

Lee JS, Collins KM, Brown AL, Lee CH, Chung JH (2000) hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature 404: 201-204

Lewis TG, Nydorf ED (2006) Intralesional bleomycin for warts: a review. Journal of Drugs in Dermatology 5: 499-504

Li GM (2007) Mechanisms and functions of DNA mismatch repair. Cell Research 18: 85-98

Li X, Stith CM, Burgers PM, Heyer W-D (2009) PCNA is required for initiation of recombination-associated DNA synthesis by DNA polymerase δ. Molecular Cell 36: 704-713

Liao S, Toczylowski T, Yan H (2008) Identification of the Xenopus DNA2 protein as a major nuclease for the 5′→ 3′ strand-specific processing of DNA ends. Nucleic Acids Research 36: 6091-6100

Liao S, Toczylowski T, Yan H (2011) Mechanistic analysis of Xenopus EXO1's function in 5′-strand resection at DNA double-strand breaks. Nucleic Acids Research 39: 5967-5977

Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat ML, Gyorki DE, Ward T, Partanen A, Feleppa F, Huschtscha LI, Thorne HJ, Fox SB, Yan M, French JD, Brown MA, Smyth GK, Visvader JE, Lindeman GJ (2009) Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nature Medicine 15: 907-913

Lipkowitz S, Weissman AM (2011) RINGs of good and evil: RING finger ubiquitin ligases at the crossroads of tumour suppression and oncogenesis. Nature Reviews Cancer 11: 629-643

Liu S, Ginestier C, Charafe-Jauffret E, Foco H, Kleer CG, Merajver SD, Dontu G, Wicha MS (2008) BRCA1 regulates human mammary stem/progenitor cell fate. Proceedings of the National Academy of Sciences of the United States of America 105: 1680-1685

Liu Z, Wu J, Yu X (2007) CCDC98 targets BRCA1 to DNA damage sites. Nature Structural & Molecular Biology 14: 716–720

Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT, Perou CM (2005) Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Modern Pathology 19: 264-271

Loffler H, Lukas J, Bartek J, Kramer A (2006) Structure meets function--centrosomes, genome maintenance and the DNA damage response. Experimental Cell Research 312: 2633-2640

Longley DB, Harkin DP, Johnston PG (2003) 5-fluorouracil: mechanisms of action and clinical strategies. Nature Reviews Cancer 3: 330-338

Longley MJ, Pierce AJ, Modrich P (1997) DNA polymerase δ is required for human mismatch repair in vitro. Journal of Biological Chemistry 272: 10917-10921

Lopez-Girona A, Furnari B, Mondesert O, Russell P (1999) Nuclear localization of Cdc25 is regulated by DNA damage and a 14-3-3 protein. Nature 397: 172-175

Lord CJ, Ashworth A (2012) The DNA damage response and cancer therapy. Nature 481: 287-294

Lord CJ, Ashworth A (2013) Mechanisms of resistance to therapies targeting BRCA-mutant cancers. Nature Medicine 19: 1381-1388

Lou Z, Chini CC, Minter-Dykhouse K, Chen J (2003) Mediator of DNA damage checkpoint protein 1 regulates BRCA1 localization and phosphorylation in DNA damage checkpoint control. The Journal of Biological Chemistry 278: 13599-13602

Luo T, Yu J, Nguyen J, Wang C-R, Bristow RG, Jaffray DA, Zhou XZ, Lu KP, Lu Q-B (2012) Electron transfer-based combination therapy of cisplatin with tetramethyl-p-phenylenediamine for ovarian, cervical, and lung cancers. Proceedings of the National Academy of Sciences of the United States of America 109: 10175-10180

Ma Y, Lu H, Tippin B, Goodman MF, Shimazaki N, Koiwai O, Hsieh C-L, Schwarz K, Lieber MR (2004) A biochemically defined system for mammalian nonhomologous DNA end joining. Molecular Cell 16: 701-713

Ma Y, Pannicke U, Schwarz K, Lieber MR (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V (D) J recombination. Cell 108: 781-794

Mahaney B, Meek K, Lees-Miller S (2009) Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochemical Journal 417: 639-650

Malhotra GK, Zhao X, Band H, Band V (2010) Histological, molecular and functional subtypes of breast cancers. Cancer Biology & Therapy 10: 955-960

Maloisel L, Fabre F, Gangloff S (2008) DNA polymerase δ is preferentially recruited during homologous recombination to promote heteroduplex DNA extension. Molecular and Cellular Biology 28: 1373-1382

Marmot MG, Altman DG, Cameron DA, Dewar JA G Thompson SG, Wilcox M (2012) The benefits and harms of breast cancer screening: an independent review. Lancet 380: 1778

Manke IA, Lowery DM, Nguyen A, Yaffe MB (2003) BRCT repeats as phosphopeptide-binding modules involved in protein targeting. Science 302: 636-639

Margolese RG, Bernard Fisher, Gabriel N Hortobagyi, and William D Bloomer (2000) Neoplasms of the Breast, Hamilton, Ontario: B.C. Decker.

Marteijn JA, Lans H, Vermeulen W, Hoeijmakers JH (2014) Understanding nucleotide excision repair and its roles in cancer and ageing. Nature Reviews Molecular Cell Biology 15: 465-481

Martin LJ (2008) DNA damage and repair: relevance to mechanisms of neurodegeneration. Journal of Neuropathology & Experimental Neurology 67: 377-387

Matos J, West SC (2014) Holliday junction resolution: regulation in space and time. DNA Repair (Amst) 19: 176-181

Mattiroli F, Vissers JH, van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, Marteijn JA, Sixma TK (2012) RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell 150: 1182-1195

McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A (2006) Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Research 66: 8109-8115

McVey M, Lee SE (2008) MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. Trends in Genetics 24: 529-538

Meek K, Dang V, Lees-Miller SP (2008) DNA-PK: the means to justify the ends? Advances in Immunology 99: 33-58

Meijers-Heijboer H, van den Ouweland A, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, Hollestelle A, Houben M, Crepin E, van Veghel-Plandsoen M (2002) Low-penetrance susceptibility to breast cancer due to CHEK2* 1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nature Genetics 31: 55-59

Meindl A, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, Freund M, Lichtner P, Hartmann L, Schaal H (2010) Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nature Genetics 42: 410-414

Melino G (2011) p63 is a suppressor of tumorigenesis and metastasis interacting with mutant p53. Cell Death and Differentiation 18: 1487-1499

Menear KA, Adcock C, Boulter R, Cockcroft XL, Copsey L, Cranston A, Dillon KJ, Drzewiecki J, Garman S, Gomez S, Javaid H, Kerrigan F, Knights C, Lau A, Loh VM, Jr., Matthews IT, Moore S, O'Connor MJ, Smith GC, Martin NM (2008) 4-[3-(4-cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin- 1-one: a novel bioavailable inhibitor of poly(ADP-ribose) polymerase-1. Journal of Medicinal Chemistry 51: 6581-6591

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71

Mimitou EP, Symington LS (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 455: 770-774

Moller P, Borg A, Evans DG, Haites N, Reis MM, Vasen H, Anderson E, Steel CM, Apold J, Goudie D, Howell A, Lalloo F, Maehle L, Gregory H, Heimdal K (2002) Survival in prospectively ascertained familial breast cancer: analysis of a series stratified by

tumour characteristics, BRCA mutations and oophorectomy. International Journal of Cancer 101: 555-559

Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R, Mackay A, Grigoriadis A, Tutt A, Ashworth A, Reis-Filho JS, Smalley MJ (2010) BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. Cell Stem Cell 7: 403-417

Morris JR, Solomon E (2004) BRCA1: BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. Human Molecular Genetics 13: 807-817

Mullan P, Quinn J, Harkin D (2006) The role of BRCA1 in transcriptional regulation and cell cycle control. Oncogene 25: 5854-5863

Muniandy PA, Liu J, Majumdar A, Liu ST, Seidman MM (2010) DNA interstrand crosslink repair in mammalian cells: step by step. Critical Reviews in Biochemistry and Molecular Biology 45: 23-49

Naderi A, Teschendorff A, Barbosa-Morais N, Pinder S, Green A, Powe D, Robertson J, Aparicio S, Ellis I, Brenton J (2006) A gene-expression signature to predict survival in breast cancer across independent data sets. Oncogene 26: 1507-1516

Nakamura K, Kogame T, Oshiumi H, Shinohara A, Sumitomo Y, Agama K, Pommier Y, Tsutsui KM, Tsutsui K, Hartsuiker E, Ogi T, Takeda S, Taniguchi Y (2010) Collaborative action of Brca1 and CtIP in elimination of covalent modifications from double-strand breaks to facilitate subsequent break repair. PLoS Genetics 6: e1000828

Narod SA, Foulkes WD (2004) BRCA1 and BRCA2: 1994 and beyond. Nature Reviews Cancer 4: 665-676

National Cancer Institute (2014) Breast Cancer Treatment (PDQ®). Available online. Last accessed April 27, 2015

Negrini S, Gorgoulis VG, Halazonetis TD (2010) Genomic instability--an evolving hallmark of cancer. Nature Reviews Molecular Cell Biology 11: 220-228

Nevins JR (2001) The Rb/E2F pathway and cancer. Human Molecular Genetics 10: 699-703

Nimonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC (2008). Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. Proceedings of the National Academy of Sciences of the United States of America 105: 16906–16911

Nishikawa H, Ooka S, Sato K, Arima K, Okamoto J, Klevit RE, Fukuda M, Ohta T (2004) Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1-BARD1 ubiquitin ligase. Journal of Biological Chemistry 279: 3916-3924

Nitiss JL (2009) Targeting DNA topoisomerase II in cancer chemotherapy. Nature Reviews Cancer 9: 338-350

Noll DM, Mason TM, Miller PS (2006) Formation and repair of interstrand cross-links in DNA. Chemical Reviews 106: 277-301

Norquist B, Wurz KA, Pennil CC, Garcia R, Gross J, Sakai W, Karlan BY, Taniguchi T, Swisher EM (2011) Secondary somatic mutations restoring BRCA1/2 predict

chemotherapy resistance in hereditary ovarian carcinomas. Journal of Clinical Oncology 29: 3008-3015

O'Connell MJ, Walworth NC, Carr AM (2000) The G2-phase DNA-damage checkpoint. Trends in Cell Biology 10: 296-303

O'Donovan A, Davies AA, Moggs JG, West SC, Wood RD (1994) XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. Nature 371: 432 - 435

Olopade OI, Grushko TA, Nanda R, Huo D (2008) Advances in breast cancer: pathways to personalized medicine. Clinical Cancer Research 14: 7988-7999

Pageau GJ, Lawrence JB (2006) BRCA1 foci in normal S-phase nuclei are linked to interphase centromeres and replication of pericentric heterochromatin. Journal of Cell Biology 175: 693-701

Pan MR, Peng G, Hung WC, Lin SY (2011) Monoubiquitination of H2AX protein regulates DNA damage response signaling. Journal of Biological Chemistry 286: 28599-28607

Paull TT, Gellert M (1998) The 3' to 5' exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. Molecular Cell 1: 969-979

Pegg AE (1990) Mammalian O6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. Cancer Research 50: 6119-6129

Pemovska T, Kontro M, Yadav B, Edgren H, Eldfors S, Szwajda A, Almusa H, Bespalov MM, Ellonen P, Elonen E, Gjertsen BT, Karjalainen R, Kulesskiy E, Lagström S, Lehto A, Leppä S, Lundán T, Majumder MM, Marti JM, Mattila P, Murumägi A, Mustjoki S, Palva A, Parsons A, Pirttinen T, Rämet ME, Suvela M, Turunen L, Västrik I, Wolf M, Knowles J, Aittokallio T, Heckman CA, Porkka K, Kallioniemi O, Wennerberg K (2013) Individualized systems medicine strategy to tailor treatments for patients with chemorefractory acute myeloid leukemia. Cancer Discovery 3:1416-1429

Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA (2000) Molecular portraits of human breast tumours. Nature 406: 747-752

Petermann E, Woodcock M, Helleday T (2010) Chk1 promotes replication fork progression by controlling replication initiation. Proceedings of the National Academy of Sciences of the United States of America 107: 16090-16095

Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P (2002) Tobacco smoke carcinogens, DNA damage and p 53 mutations in smoking-associated cancers. Oncogene 21: 7435-7451

Pfeiffer P, Goedecke W, Obe G (2000) Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations. Mutagenesis 15: 289-302

Plo I, Liao ZY, Barcelóa JM, Kohlhagen G, Caldecott KW, Weinfeld M, Pommier Y (2003) Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions. DNA Repair 2: 1087-1100

Polanowska J, Martin JS, Garcia-Muse T, Petalcorin MI, Boulton SJ (2006) A conserved pathway to activate BRCA1-dependent ubiquitylation at DNA damage sites. The EMBO Journal 25: 2178-2188

Polo SE, Jackson SP (2011) Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. Genes & Development 25: 409-433

Poulogiannis G, Frayling IM, Arends MJ (2010) DNA mismatch repair deficiency in sporadic colorectal cancer and Lynch syndrome. Histopathology 56: 167-179

Poumpouridou N, Kroupis C (2012) Hereditary breast cancer: beyond BRCA genetic analysis; PALB2 emerges. Clinical Chemistry and Laboratory Medicine 50: 423-434

Povirk LF, Shuker DE (1994) DNA damage and mutagenesis induced by nitrogen mustards. Mutation Research/Reviews in Genetic Toxicology 318: 205-226

Prat A, Adamo B, Fan C, Peg V, Vidal M, Galvan P, Vivancos A, Nuciforo P, Palmer HG, Dawood S, Rodon J, Ramony Cajal S, Del Campo JM, Felip E, Tabernero J, Cortes J (2013) Genomic analyses across six cancer types identify basal-like breast cancer as a unique molecular entity. Scientific Reports 3: 3544

Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X, Perou CM (2010) Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Research 12: R68

Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Easton DF, Stratton MR (2007) PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nature Genetics 39: 165-167

Rakha EA, Ellis IO (2009) Triple-negative/basal-like breast cancer: review. Pathology 41: 40-47

Rakha EA, Reis-Filho JS, Ellis IO (2008) Basal-like breast cancer: a critical review. Journal of Clinical Oncology 26: 2568-2581

Ratajska M, Antoszewska E, Piskorz A, Brozek I, Borg Å, Kusmierek H, Biernat W, Limon J (2012) Cancer predisposing BARD1 mutations in breast–ovarian cancer families. Breast Cancer Research and Treatment 131: 89-97

Reczek CR, Szabolcs M, Stark JM, Ludwig T, Baer R (2013) The interaction between CtIP and BRCA1 is not essential for resection-mediated DNA repair or tumor suppression. Journal of Cell Biology 201: 693-707

Roskoski R, Jr. (2012) MEK1/2 dual-specificity protein kinases: structure and regulation. Biochemical and Biophysical Research Communications 417: 5-10

Rottenberg S, Jaspers JE, Kersbergen A, van der Burg E, Nygren AO, Zander SA, Derksen PW, de Bruin M, Zevenhoven J, Lau A, Boulter R, Cranston A, O'Connor MJ, Martin NM, Borst P, Jonkers J (2008) High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proceedings of the National Academy of Sciences of the United States of America 105: 17079-17084

Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG (2010) PARP inhibition: PARP1 and beyond. Nature Reviews Cancer 10: 293-301

Roy R, Chun J, Powell SN (2011) BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nature Reviews Cancer 12: 68-78

Roy V, Perez EA (2009) Beyond trastuzumab: small molecule tyrosine kinase inhibitors in HER-2–positive breast cancer. The Oncologist 14: 1061-1069

Russnes HG, Navin N, Hicks J, Borresen-Dale AL (2011) Insight into the heterogeneity of breast cancer through next-generation sequencing. Journal of Clinical Investigation 121: 3810-3818

Sabatier R, Finetti P, Guille A, Adelaide J, Chaffanet M, Viens P, Birnbaum D, Bertucci F (2014) Claudin-low breast cancers: clinical, pathological, molecular and prognostic characterization. Molecular Cancer 13: 228

Sakai W, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, Villegas E, Jacquemont C, Farrugia DJ, Couch FJ, Urban N, Taniguchi T (2008) Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 451: 1116-1120

Sampath D, Shi Z, Plunkett W (2002) Inhibition of cyclin-dependent kinase 2 by the Chk1-Cdc25A pathway during the S-phase checkpoint activated by fludarabine: dysregulation by 7-hydroxystaurosporine. Molecular Pharmacology 62: 680-688

San Filippo J, Sung P, Klein H (2008) Mechanism of eukaryotic homologous recombination. Annual Review of Biochemistry 77: 229-257

Sancar A, Lindsey-Boltz LA, Ünsal-Kaçmaz K, Linn S (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annual Review of Biochemistry 73: 39-85

Sankaran S, Starita LM, Groen AC, Ko MJ, Parvin JD (2005) Centrosomal microtubule nucleation activity is inhibited by BRCA1-dependent ubiquitination. Molecular and Cellular Biology 25: 8656-8668

Sankaran S, Starita LM, Simons AM, Parvin JD (2006) Identification of domains of BRCA1 critical for the ubiquitin-dependent inhibition of centrosome function. Cancer Research 66: 4100-4107

Santoro A, Bonadonna G, Valagussa P, Zucali R, Viviani S, Villani F, Pagnoni A, Bonfante V, Musumeci R, Crippa F (1987) Long-term results of combined chemotherapy-radiotherapy approach in Hodgkin's disease: superiority of ABVD plus radiotherapy versus MOPP plus radiotherapy. Journal of Clinical Oncology 5: 27-37

Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP (2007) Human CtIP promotes DNA end resection. Nature 450: 509-514

Schlacher K, Wu H, Jasin M (2012) A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. Cancer Cell 22: 106-116

Schlegel BP, Jodelka FM, Nunez R (2006) BRCA1 promotes induction of ssDNA by ionizing radiation. Cancer Research 66: 5181-5189

Schreiber V, Amé J-C, Dollé P, Schultz I, Rinaldi B, Fraulob V, Ménissier-de Murcia J, de Murcia G (2002) Poly (ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. Journal of Biological Chemistry 277: 23028-23036

Schreiber V, Dantzer F, Ame JC, de Murcia G (2006) Poly(ADP-ribose): novel functions for an old molecule. Nature Reviews Molecular Cell Biology 7: 517-528

Schultz LB, Chehab NH, Malikzay A, DiTullio R, Stavridi ES, Halazonetis TD (2000) The DNA damage checkpoint and human cancer. In Cold Spring Harbor Symposia on Quantitative Biology Vol. 65, pp 489-498

Scully R, Chen J, Ochs RL, Keegan K, Hoekstra M, Feunteun J, Livingston DM (1997b) Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. Cell 90: 425-435

Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM (1997a) Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 88: 265-275

Seiple L, Jaruga P, Dizdaroglu M, Stivers JT (2006) Linking uracil base excision repair and 5-fluorouracil toxicity in yeast. Nucleic Acids Research 34: 140-151

Shabbeer S, Omer D, Berneman D, Weitzman O, Alpaugh A, Pietraszkiewicz A, Metsuyanim S, Shainskaya A, Papa MZ, Yarden RI (2013) BRCA1 targets G2/M cell cycle proteins for ubiquitination and proteasomal degradation. Oncogene 32: 5005-5016

Shakya R, Szabolcs M, McCarthy E, Ospina E, Basso K, Nandula S, Murty V, Baer R, Ludwig T (2008) The basal-like mammary carcinomas induced by Brca1 or Bard1 inactivation implicate the BRCA1/BARD1 heterodimer in tumor suppression. Proceedings of the National Academy of Sciences of the United States of America 105: 7040-7045

Shao Z, Davis AJ, Fattah KR, So S, Sun J, Lee K-J, Harrison L, Yang J, Chen DJ (2012) Persistently bound Ku at DNA ends attenuates DNA end resection and homologous recombination. DNA Repair 11: 310-316

Shrivastav M, De Haro LP, Nickoloff JA (2007) Regulation of DNA double-strand break repair pathway choice. Cell Research 18: 134-147

Shroff R, Arbel-Eden A, Pilch D, Ira G, Bonner WM, Petrini JH, Haber JE, Lichten M (2004) Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Current Biology 14: 1703-1711

Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics, 2014. CA: A Cancer Journal for Clinicians 64: 9-29

Sijbers AM, de Laat WL, Ariza RR, Biggerstaff M, Wei Y-F, Moggs JG, Carter KC, Shell BK, Evans E, de Jong MC (1996) Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. Cell 86: 811-822

Simons AM, Horwitz AA, Starita LM, Griffin K, Williams RS, Glover JM, Parvin JD (2006) BRCA1 DNA-binding activity is stimulated by BARD1. Cancer Research 66: 2012-2018

Singletary SE, Greene FL (2003) Revision of breast cancer staging: the 6th edition of the TNM Classification. Seminars in Surgical Oncology 21: 53-59

Smalley M, Ashworth A (2003) Stem cells and breast cancer: A field in transit. Nature Reviews Cancer 3: 832-844

Smalley MJ, Reis-Filho JS, Ashworth A (2008) BRCA1 and stem cells: tumour typecasting. Nature Cell Biology 10: 377-379

Smart C, Wronski A, French J, Edwards S, Asselin-Labat M, Waddell N, Peters K, Brewster B, Brooks K, Simpson K (2010) Analysis of Brca1-deficient mouse mammary glands reveals reciprocal regulation of Brca1 and c-kit. Oncogene 30: 1597-1607

Smith J, Mun Tho L, Xu N, Gillespie DA (2010) The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. Advances in Cancer Research 108: 73-112

Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N (2006) BRAF mutation predicts sensitivity to MEK inhibition. Nature 439: 358-362

Somasundaram K, Zhang H, Zeng YX, Houvras Y, Peng Y, Zhang H, Wu GS, Licht JD, Weber BL, El-Deiry WS (1997) Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CiP1. Nature 389: 187-190

Sørlie T, Tibshirani R, Parker J, Hastie T, Marron J, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proceedings of the National Academy of Sciences 100: 8418-8423

Sotiriou C, Neo S-Y, McShane LM, Korn EL, Long PM, Jazaeri A, Martiat P, Fox SB, Harris AL, Liu ET (2003) Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proceedings of the National Academy of Sciences of the United States of America 100: 10393-10398

Sotiriou C, Pusztai L (2009) Gene-expression signatures in breast cancer. New England Journal of Medicine 360: 790-800

Soule HD, Maloney TM, Wolman SR, Peterson WD Jr, Brenz R, McGrath CM, Russo J, Pauley RJ, Jones RF, Brooks SC (1990) Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. Cancer Research 50: 6075-6086

Spycher C, Miller ES, Townsend K, Pavic L, Morrice NA, Janscak P, Stewart GS, Stucki M (2008) Constitutive phosphorylation of MDC1 physically links the MRE11–RAD50–NBS1 complex to damaged chromatin. Journal of Cell Biology 181: 227-240

Staff S, Nupponen NN, Borg Å, Isola JJ, Tanner MM (2000) Multiple copies of mutant BRCA1 and BRCA2 alleles in breast tumors from germ-line mutation carriers. Genes, Chromosomes and Cancer 28: 432-442

Starita LM, Horwitz AA, Keogh M-C, Ishioka C, Parvin JD, Chiba N (2005) BRCA1/BARD1 ubiquitinate phosphorylated RNA polymerase II. Journal of Biological Chemistry 280: 24498-24505

Starita LM, Machida Y, Sankaran S, Elias JE, Griffin K, Schlegel BP, Gygi SP, Parvin JD (2004) BRCA1-dependent ubiquitination of γ-tubulin regulates centrosome number. Molecular and Cellular Biology 24: 8457-8466

Steffensen K, Waldstrøm M, Jeppesen U, Brandslund I, Jakobsen A (2008) Prediction of response to chemotherapy by ERCC1 immunohistochemistry and ERCC1 polymorphism in ovarian cancer. International Journal of Gynecological Cancer 18: 702-710

Stevens EV, Raffeld M, Espina V, Kristensen GB, Trope CG, Kohn EC, Davidson B (2005) Expression of xeroderma pigmentosum A protein predicts improved outcome in metastatic ovarian carcinoma. Cancer 103: 2313-2319

Stewart GS, Wang B, Bignell CR, Taylor AM, Elledge SJ (2003) MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature 421: 961-966

Stracker TH, Petrini JH (2011) The MRE11 complex: starting from the ends. Nature Reviews Molecular Cell Biology 12: 90-103

Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP (2005) MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell 123: 1213–1226

Su X, Chakravarti D, Flores ER (2013) p63 steps into the limelight: crucial roles in the suppression of tumorigenesis and metastasis. Nature Reviews Cancer 13: 136-143

Sugasawa K, Okamoto T, Shimizu Y, Masutani C, Iwai S, Hanaoka F (2001) A multistep damage recognition mechanism for global genomic nucleotide excision repair. Genes & Development 15: 507-521

Sung P, Robberson DL (1995) DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. Cell 82: 453-461

Swisher EM, Sakai W, Karlan BY, Wurz K, Urban N, Taniguchi T (2008) Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. Cancer Research 68: 2581-2586

Sy SM, Huen MS, Chen J (2009) PALB2 is an integral component of the BRCA complex required for homologous recombination repair. Proceedings of the National Academy of Sciences of the United States of America 106: 7155-7160

Symington LS, Gautier J (2011) Double-strand break end resection and repair pathway choice. Annual Review of Genetics 45: 247-271

Tassone P, Tagliaferri P, Perricelli A, Blotta S, Quaresima B, Martelli ML, Goel A, Barbieri V, Costanzo F, Boland CR, Venuta S (2003) BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells. British Journal of Cancer 88: 1285-1291

Tavassoli FA, Devilee, P. (2003) Pathology and Genetics of Tumours of the Breast and Female Genital Organs. IARC WHO Classification of Tumours, No 4

Thakur S, Zhang HB, Peng Y, Le H, Carroll B, Ward T, Yao J, Farid LM, Couch FJ, Wilson RB (1997) Localization of BRCA1 and a splice variant identifies the nuclear localization signal. Molecular and Cellular Biology 17: 444-452

Thibodeau SN, French AJ, Roche PC, Cunningham JM, Tester DJ, Lindor NM, Moslein G, Baker SM, Liskay RM, Burgart LJ (1996) Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Research 56: 4836-4840

Tibbetts RS, Cortez D, Brumbaugh KM, Scully R, Livingston D, Elledge SJ, Abraham RT (2000) Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. Genes & Development 14: 2989-3002

Toft DJ, Cryns VL (2010) Minireview: Basal-like breast cancer: from molecular profiles to targeted therapies. Molecular Endocrinology 25: 199-211

Truong LN, Li Y, Shi LZ, Hwang PY-H, He J, Wang H, Razavian N, Berns MW, Wu X (2013) Microhomology-mediated End Joining and Homologous Recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America 110: 7720-7725

Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, Seal S, Ghoussaini M, Hines S, Healey CS (2010) Genome-wide association study identifies five new breast cancer susceptibility loci. Nature Genetics 42: 504-507

Turner N, Tutt A, Ashworth A (2004) Hallmarks of 'BRCAness' in sporadic cancers. Nature Reviews Cancer 4: 814-819

Turner NC, Lord CJ, Iorns E, Brough R, Swift S, Elliott R, Rayter S, Tutt AN, Ashworth A (2008) A synthetic lethal siRNA screen identifying genes mediating sensitivity to a PARP inhibitor. The EMBO Journal 27: 1368-1377

Turner NC, Reis-Filho JS (2013) Tackling the diversity of triple-negative breast cancer. Clinical Cancer Research 19: 6380-6388

Turner NC, Tutt AN (2012) Platinum chemotherapy for BRCA1-related breast cancer: do we need more evidence? Breast Cancer Research 14: 115

van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ (2002) A gene-expression signature as a predictor of survival in breast cancer. New England Journal of Medicine 347: 1999-2009

van den Heuvel S, Dyson NJ (2008) Conserved functions of the pRB and E2F families. Nature Reviews Molecular Cell Biology 9: 713-724

Vandenberg CJ, Gergely F, Ong CY, Pace P, Mallery DL, Hiom K, Patel KJ (2003) BRCA1-independent ubiquitination of FANCD2. Molecular Cell 12: 247-254

van't Veer LJ, Dai H, Van De Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415: 530-536

Veer P, Kampman E (2007) Food, nutrition, physical activity and the prevention of cancer: a global perspective. Washington, DC: World Cancer Research Fund/American Institute for Cancer Research

Venkitaraman AR (2002) Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 108: 171-182

Venkitaraman AR (2009) Linking the cellular functions of BRCA genes to cancer pathogenesis and treatment. Annual Review of Pathological Mechanical Disease 4: 461-487

Viale G (2012) The current state of breast cancer classification. Annals of Oncology 23: x207-x210

Vitale I, Galluzzi L, Castedo M, Kroemer G (2011) Mitotic catastrophe: a mechanism for avoiding genomic instability. Nature Reviews Molecular Cell Biology 12: 385-392

Vousden KH, Prives C (2009) Blinded by the light: the growing complexity of p53. Cell 137: 413-431

Walsh T, King M-C (2007) Ten genes for inherited breast cancer. Cancer Cell 11: 103-105

Wang B, Elledge SJ (2007) Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brca1/Brcc36 complex in response to DNA damage. Proceedings of the National Academy of Sciences of the United States of America 104: 20759–20763

Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP, Elledge SJ (2007) Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science 316: 1194-1198

Wang JC (2002) Cellular roles of DNA topoisomerases: a molecular perspective. Nature Reviews Molecular Cell Biology 3: 430-440

Wang M, Wu W, Wu W, Rosidi B, Zhang L, Wang H, Iliakis G (2006) PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. Nucleic Acids Research 34: 6170-6182

Watson CJ, Khaled WT (2008) Mammary development in the embryo and adult: a journey of morphogenesis and commitment. Development 135: 995-1003

Weigelt B, Reis-Filho JS (2009) Histological and molecular types of breast cancer: is there a unifying taxonomy? Nature Reviews Clinical Oncology 6: 718-730

Weinberg RA (2007) pRb and control of the cell cycle clock. In: The Biology of Cancer. New York: Garland Science: 255-307

Weinstock DM, Brunet E, Jasin M (2007) Formation of NHEJ-derived reciprocal chromosomal translocations does not require Ku70. Nature Cell Biology 9: 978-981

Welcsh PL, King M-C (2001) BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. Human Molecular Genetics 10: 705-713

Wilson MA, Kwon Y, Xu Y, Chung W-H, Chi P, Niu H, Mayle R, Chen X, Malkova A, Sung P (2013) Pif1 helicase and Pol [dgr] promote recombination-coupled DNA synthesis via bubble migration. Nature 502: 393-396

Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D (1994) Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 265: 2088-2090

Wu-Baer F, Lagrazon K, Yuan W, Baer R (2003) The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. Journal of Biological Chemistry. 278: 34743-34746

Wu L, Hickson ID (2003) The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 426: 870-874

Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang M-CW, Hwang L-Y, Bowcock AM, Baer R (1996) Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nature Genetics 14: 430-440

Wyatt HD, Sarbajna S, Matos J, West SC (2013) Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. Molecular Cell 52: 234-247

Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM (2006) Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. Molecular Cell 22: 719-729

Xia Y, Pao GM, Chen H-W, Verma IM, Hunter T (2003) Enhancement of BRCA1 E3 ubiquitin ligase activity through direct interaction with the BARD1 protein. Journal of Biological Chemistry 278: 5255-5263

Xiao Z, Chen Z, Gunasekera AH, Sowin TJ, Rosenberg SH, Fesik S, Zhang H (2003) Chk1 mediates S and G2 arrests through Cdc25A degradation in response to DNA-damaging agents. The Journal of Biological Chemistry 278: 21767-21773

Xu B, Kim S, Kastan MB (2001) Involvement of Brca1 in S-phase and G(2)-phase checkpoints after ionizing irradiation. Molecular and Cellular Biology 21: 3445-3450

Xu B, O'Donnell AH, Kim ST, Kastan MB (2002) Phosphorylation of serine 1387 in Brca1 is specifically required for the Atm-mediated S-phase checkpoint after ionizing irradiation. Cancer Research 62: 4588-4591

Xu X, Qiao W, Linke SP, Cao L, Li WM, Furth PA, Harris CC, Deng CX (2001) Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. Nature Genetics 28: 266-271

Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX (1999) Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Molecular Cell 3: 389-395

Yamamori T, Yasui H, Yamazumi M, Wada Y, Nakamura Y, Nakamura H, Inanami O (2012) Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint. Free Radical Biology and Medicine 53: 260-270

Yan J, Kim YS, Yang XP, Li LP, Liao G, Xia F, Jetten AM (2007) The ubiquitin-interacting motif containing protein RAP80 interacts with BRCA1 and functions in DNA damage repair response. Cancer Research 67: 6647-6656

Yano Ki, Morotomi-Yano K, Wang SY, Uematsu N, Lee KJ, Asaithamby A, Weterings E, Chen DJ (2008) Ku recruits XLF to DNA double-strand breaks. EMBO Reports 9: 91-96

Yarden RI, Pardo-Reoyo S, Sgagias M, Cowan KH, Brody LC (2002) BRCA1 regulates the G2/M checkpoint by activating Chk1 kinase upon DNA damage. Nature Genetics 30: 285-289

Yaswen P, Stampfer MR (2002) Molecular changes accompanying senescence and immortalization of cultured human mammary epithelial cells. The International Journal of Biochemistry & Cell Biology 34: 1382-1394

Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY, Qin J (2002) SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. Genes & Development 16: 571-582

Yu X, Chen J (2004) DNA damage-induced cell cycle checkpoint control requires CtIP, a phosphorylation-dependent binding partner of BRCA1 C-terminal domains. Molecular and Cellular Biology 24: 9478-9486

Yu X, Chini CCS, He M, Mer G, Chen J (2003) The BRCT domain is a phospho-protein binding domain. Science 302: 639-642

Yu X, Fu S, Lai M, Baer R, Chen J (2006) BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. Genes & Development 20: 1721-1726

Yu X, Jacobs SA, West SC, Ogawa T, Egelman EH (2001) Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA. Proceedings of the National Academy of Sciences of the United States of America 98: 8419-8424

Yuan J, Chen J (2010) MRE11-RAD50-NBS1 complex dictates DNA repair independent of H2AX. Journal of Biological Chemistry 285: 1097-1104

Yun MH, Hiom K (2009) CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature 459: 460-463

Zhang F, Ma J, Wu J, Ye L, Cai H, Xia B, Yu X (2009) PALB2 links BRCA1 and BRCA2 in the DNA-damage response. Current Biology 19: 524-529

Zhang J, Willers H, Feng Z, Ghosh JC, Kim S, Weaver DT, Chung JH, Powell SN, Xia F (2004) Chk2 phosphorylation of BRCA1 regulates DNA double-strand break repair. Molecular and Cellular Biology 24: 708-718

Zhang Y, Yuan F, Presnell SR, Tian K, Gao Y, Tomkinson AE, Gu L, Li G-M (2005) Reconstitution of 5′-directed human mismatch repair in a purified system. Cell 122: 693-705

Zhao GY, Sonoda E, Barber LJ, Oka H, Murakawa Y, Yamada K, Ikura T, Wang X, Kobayashi M, Yamamoto K, Boulton SJ, Takeda S (2007) A critical role for the ubiquitin-conjugating enzyme Ubc13 in initiating homologous recombination. Molecular Cell 25: 663-675

Zhao Y, Thomas HD, Batey MA, Cowell IG, Richardson CJ, Griffin RJ, Calvert AH, Newell DR, Smith GC, Curtin NJ (2006) Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441. Cancer Research 66: 5354-5362